Study of Durif (*Vitis vinifera* L.) berry ripening phases in relationship to wine styles

by

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Summary

The wines of Durif (Petite Sirah) are known for their exceptionally dark colour and astringency (mouth feel). The goal of this research project was to assess Durif wine styles, profiles and diversity using sequential harvest. Grapes were harvested from a Durif vineyard in Paarl, South Africa, at different stages of ripeness on different dates (sequential harvesting) influencing the grape composition, which, in turn, influences wine composition and sensory attributes. There is not only one optimal harvest date for a vineyard or specific site and several harvest dates could be adequate. This research project spanned the 2012 and 2013 vintages. The 2012 was used as a diagnostic year to characterize the vineyard plot and the options for harvest timing.

Sequential harvesting was used to determine if Durif follows a berry aromatic evolution (Deloire, 2011). The berry aromatic evolution describes wine styles, which can be achieved by determining harvest time/period using sugar loading as a berry physiological indicator. These wine styles include a fresh fruit (FF) style, a neutral, spicy or pre-mature fruit style, a mature fruit (MF) style and later an over ripe style (OR). One of the major findings of this research project was calibrating the model of berry sugar accumulation for Durif to determine picking windows/dates for specific wine styles.

The wines made from sequentially harvested grapes were sensorially assessed using the citation frequency method (Campo *et al.,* 2010).The sensory analysis was used to find the sensory attributes of wines from two harvest time points corresponding to fresh fruit and mature fruit style wines. The main aromatic attributes associated with Durif wines in South Africa were identified. Descriptive analysis was used to rate the intensity of astringency, bitterness, sweetness, sourness and alcohol perception of the wines and how it changes with harvest timing

Berry composition was analysed (fructose, glucose, tartaric acid, malic acid, total anthocyanins and total phenolics) and it was shown that there were significant changes in berry composition during ripening. The evolution of berry composition during the ripening period highlights the importance of optimization of the picking date. The wine composition, however, is not directly related to grape composition as it is compounded by the impact of extraction during fermentation (with increasing levels of ethanol with increasing sugar levels when using sequential harvesting) on tannin and anthocyanin extractability and yeast metabolism (Gil *et al*., 2015, Fournand *et al.,* 2006). Must composition (pH, TA, Brix) was monitored and showed significant differences between early and later harvest dates. Wine composition of each sequentially harvested wine was analysed.

From the trends observed in the frequency of citation method it is suggested that the berry aromatic ripening sequence for Durif exists and fresh or mature fruit style wines can be made. Harvest timing could be defined as picking ten days after the keypoint to produce fresh fruit style wines and twenty-three days to produce mature fruit style wines according to the results of the 2013 vintage.

Opsomming

Wyne afkomstig van Durif (Petite Sirah) is bekend vir hulle buitengewoon donker kleur en vrankheid (mondgevoel). Die doelwit van hierdie navorsingsprojek was om die wynstyle, -profiele en -diversiteit van Durif met behulp van opeenvolgende oes te bepaal. Die druiwe is in ’n Durif-wingerd in die Paarl, Suid-Afrika, op verskillende rypheidstadiums en op verskillende datums geoes (opeenvolgende oes), wat die druifsamestelling beïnvloed het en wat op sy beurt weer wynsamestelling en sensoriese kenmerke beïnvloed het. Daar is nie net een optimale oesdatum vir ’n wingerd of spesifieke ligging nie en etlike oesdatums sou voldoende kan wees. Hierdie navorsingsprojek is in die 2012 en 2013 oesjare onderneem. Die diagnostiese jaar was 2012 en is gebruik om die wingerdblok te karakteriseer en die opsies vir die tyd van oes te bepaal.

Opeenvolgende oes is gebruik om te bepaal of Durif aromatiese evolusie in die korrel ondergaan (Deloire, 2011). Die aromatiese evolusie van die korrel beskryf wynstyle wat bekom kan word deur die oestyd/tydperk te bepaal op grond van suikerlading as ’n aanduier van korrelfisiologie. Hierdie wynstyle sluit in vars vrugte (*fresh fruit (FF)*), neutraal, speseryagtig of voorryp, volwasse vrug (*mature fruit (MF)*) en later oorryp (*overripe style (OR)*). Een van die vernaamste bevindinge van hierdie navorsingsprojek was die kalibrasie van ’n model van suikerophoping in die korrel vir Durif om die plukvensters/datums vir spesifieke wynstyle te bepaal.

Die wyne wat met die opeenvolgend geoeste druiwe gemaak is, is sensories geanaliseer d.m.v. die aanhalingsfrekwensiemetode (*citation frequency method*) (Campo *et al.,* 2010). Sensoriese analise is gebruik om die sensoriese eienskappe te bepaal van wyne afkomstig van twee spesifieke oestye wat ooreenstem met die vars vrug en volwasse vrug wynstyle. Die vernaamste aromatiese bydraers wat verband hou met Durif-wyne in Suid-Afrika is geïdentifiseer. Beskrywende analise is gebruik om die intensiteit van vrankheid, bitter, suurheid en alkoholwaarneming van die wyne te beoordeel en hoe dit met die tyd van oes verander.

Korrelsamestelling is geanaliseer (fruktose, glukose, wynsteensuur, appelsuur, totale antosianiene en totale fenoliese stowwe) en daar is getoon dat noemenswaardige veranderinge in korrelsamestelling tydens rypwording plaasgevind het. Die evolusie van korrelsamestelling tydens die rypwordingstydperk bring die belangrikheid na vore om die plukdatum te optimaliseer. Wynsamestelling hou egter nie direk verband met druifsamestelling nie, aangesien dit versterk word deur die impak van ekstraksie tydens gisting (met toenemende vlakke van etanol tesame met toenemende suikervlakke wanneer opeenvolgende oes gebruik word) op die ekstraheerbaarheid van tannien en antosianien en op gismetabolisme (Gil *et al*., 2015, Fournand *et al.,* 2006). Mossamestelling (pH, TA, Brix) is gemonitor en het noemenswaardige verskille getoon tussen die vroeër en later oesdatums. Die wynsamestelling van elke opeenvolgend geoeste wyn is geanaliseer.

Vanuit die tendense wat in die aanhalingsfrekwensiemetode waargeneem is, word daar voorgestel dat daar ’n korrel aromatiese rypwordingsopeenvolging vir Durif bestaan en dat vars en volwasse wynstyle verkry kan word. Volgens die resultate vanaf die 2013-oesjaar kan die tyd van oes gedefinieer word as pluk tien dae ná die sleutelpunt om vars wynstyle te produseer en drie-en-twintig dae daarna om volwasse wynstyle te produseer.

Biographical sketch

Stephanie Wiid – born on 30 October 1986 – completed her secondary education at Parktown High School for Girls, Johannesburg, in 2004. Stephanie is the youngest granddaughter of Niel Joubert, known for pioneering the establishment of the Stellenbosch Wine Route with Frans Malan and Spatz Sperling. With this background, it was not far-fetched for a girl from Johannesburg, far from the vineyards of Stellenbosch, to embark on a career in the wine industry.

Stephanie completed a BScAgric in Viticulture and Oenology *Cum Laude* at the University of Stellenbosch in 2008. She started working as a harvest intern at Fairview Winery in 2009. In 2012, Stephanie enrolled as a part-time student for an MScAgric in Viticulture while working at Fairview Winery in Paarl. During her time at Fairview Winery, Stephanie has progressed from assistant winemaker in 2009, to the role of winemaker in 2014. Stephanie's wine industry experience also include harvests in Bordeaux, France, in 2009 and at Nyetimber, England, in 2010.

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Preface

This thesis is presented as a compilation of six chapters.

Chapter 1	Introducing the context, goals and objectives
Chapter 2	Literature review
Chapter 3	2012 vintage – A diagnostic year
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Chapter 5	Using sequential harvesting to assess Durif wine styles using berry sugar loading as a physiological indicator
Chapter 6	General discussion and perspectives

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Chapter 1

Introducing the context, goals and objectives

Chapter 1: Introducing the context, goals and objectives

1.1 Background and context

Fairview Winery, a family owned winery situated in Paarl, South Africa, is renowned for its innovative marketing. Owner Charles Back pioneered the first plantings of Viognier in South Africa and is well-known for growing and promoting unusual varieties – Tannat, Tempranillo, Sangiovese, Grenache, Barbera, Roussanne, to name a few. Durif (or Petite Sirah) is one of the unusual varieties planted at Fairview Estate in Paarl. Fairview Wines has the largest plantings of Petite Sirah in South Africa at 9.15 of 54 hectares (SAWIS, 2015). Inspired by the Petite Sirah wines made in California, Fairview planted their first Durif vines in 2006.

The winemaking team was challenged by the high concentration of dry tannins associated with traditional winemaking methods at Fairview. Relatively unknown in South Africa, this variety providing astringent wine can be challenging. The wines have an exceptionally dark colour and can be perceived as astringent (mouth feel). This makes the wines suitable blending components contributing to the final blends' structure and colour. *"If winemakers can tame Petite Sirah's formidable tannins, the wine can be a mouthful but in a blowsy way, 'chewable,' in a manner of thinking. But it's more like liquid meat than any red wine I've ever come across, with a savory aroma of blackberries and spice"* (St. John, 2011).

At the time this research project started in 2011, the personality and characteristics of this variety and its wines as a single variety were yet to be explored in South Africa. The study in Durif wine styles arose from Fairview's desire to explore alternative solutions to tame Durif's "formidable tannins", with particular interest in the effect on the mouth feel and astringency. The tendency to pick the grapes at a late or very late ripening stage resulted in bold wines with high alcohols, often with the perception of very dry tannins. There seemed to be a tendency for the grapes to turn from "mature fruit" to "overripe" very quickly and understanding the harvest timing would be crucial in wine quality (wine flavours).

In parallel to this research project, Fairview explored various winemaking techniques. The winery started a quest for alternative winemaking solutions to deal with Durif's tannins. This included experimenting with extraction and maceration timing, as well as carbonic maceration. A micro-oxygenation programme was successfully introduced with the assistance of Vivelys (France, www.vivelys.com) with a specific focus on Durif wine styles. This led to the introduction of Durif in the blend of Fairview's *Goats do Roam Red* brand.

This research project spanned the 2012 and 2013 vintages. 2012 was used as a diagnostic year (or type of preliminary study) to characterize the vineyard plot and the options for harvest timing. In 2012 several practical problems were solved, for example, the irrigation scheduling was calibrated,

canopy management practices — such as leaf removal and lateral shoot removal — were compared in terms of cost efficiency and the first study of sequential harvesting on Durif was done in two different vigour situations.

The lessons learnt in the 2012 season were used to improve the plot layout in 2013, allowing for biological repeats in a homogenous section of the vineyard. Harvest timing was calibrated based on the wines of 2012 and winemakers' tasting descriptions. The main study of Durif and wine styles (Chapter 5) was completed over one year and, as the layout of the 2012 season is not comparable, further study is recommended to confirm the findings of 2013.

1.2 Goals and objectives

1.2.1 Durif wine profiles and harvest timing

The goal of this research project was to assess the Durif wine style, profiles and diversity using sequential harvest. Grapes were harvested at different stages of ripeness on different dates (sequential harvesting). The choice of harvest dates influences grape composition which, in turn, influences wine composition and sensory attributes. There is not only one optimal harvest date for a vineyard or specific site and several harvest dates could be adequate. The optimization thereof is dependent on the desired wine style (Coombe ,1992). Many physiological indicators can be used to determine picking date, for example, Brix (or equivalent measures of total soluble solids), pH and tartaric acid evolution and ratios, berry tasting, phenolics ripeness (Olarte Mantilla *et al.,* 2012, Boss *et al.,* 2014). In California, Petite Sirah is widely planted and better understood in the cellar. McCay Cellars use sequential harvesting on a commercial scale. Durif is picked in three batches with a three to three-and-a-half degree difference in Brix. The earlier pickings are described as "brighter blueberry, a softer, elegant style, brighter phenolics" and the later pickings "have a deeper, richer structure" (Boone, 2012).

In this research project, sequential harvesting was used to determine if Durif follows a berry aromatic sequence (BAS) (Deloire,2011) or berry aromatic evolution. The berry aromatic evolution describes wine styles, which can be achieved by determining harvest time/period using sugar loading as a berry physiological indicator. These wine styles include a fresh fruit (FF) style, a neutral, spicy or pre mature fruit style, a mature fruit (MF) style and later an over ripe style (OR). The wines were sensorially assessed using the citation frequency method (Campo *et al.,* 2010), a new method at the University of Stellenbosch, South Africa. The sensory analysis was used to find the sensory attributes of wines from two harvest time points corresponding to fresh fruit and mature fruit style wines. The goal was to provide practical solutions to determine picking time/period in order to assess the potential wine styles and to anticipate the harvest date, which can be applied commercially to try to ensure consistency in wine styles across different vintages.

1.2.2 Aromatic attributes of Durif wines

The goal was to determine the main aromatic attributes associated with Durif wines in South Africa. Durif is well known for its dark colour and tannin and is often used as a blending component. It has been described as an excellent cultivar for aging with blackberry, chocolate and black pepper, blueberry, liquorice and herbal elements (Wine-Searcher, 2015). The citation frequency method was used to identify the most highly cited attributes for each sequentially harvested wine, providing an overview for Durif wines in general. The most highly cited attributes over two vintages were identified.

1.2.3 Mouthfeel

Durif is well known for making wines which can be described as astringent and having high tannin concentration (robust tannins). Another goal of the project was to determine if the mouth feel and perception of astringency change with harvest timing. Descriptive analysis was used to rate the intensity of astringency, bitterness, sweetness, sourness and alcohol perception of the wines.

To summarize the aim of the study was to study the Durif (*Vitis vinifera* L.) berry ripening phases in relationship to wine styles. The objectives were:

- Sequential harvesting using sugar loading as a physiological indicator
- Chemical characterization of grapes and wines
- Sensory characterization of the wines

1.3 Bibliography

Boone, V., Wine Enthusiast, Taming Petite Sirah. Available: http://www.winemag.com/Web-2012/Taming-Petite-Sirah/ , 2012, 2016 (last update).

Boss, P.K., Böttcher, C. and Davies, C., 2014. Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *American Journal of Enology and Viticulture,* 65 (3), pp. 341–353.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference,* 21 (1), pp. 44–55.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. *American journal of Enology and Viticulture,* 43 (1), pp. 101.

Deloire, A., 2011. New method to determine optimal ripeness for different white wine styles. *Wineland Magazine,* May, pp. 84–87.

Olarte Mantilla, S.M., Collins, C., Iland, P.G., Johnson, T.E. and Bastian, S.E.P., 2012. Review: Berry sensory assessment: concepts and practices for assessing wine grapes sensory attributes. *Australian Journal of Grape and Wine Research,* 18 (3), pp. 245–255.

SAWIS, 2015-last update, SAWIS. Available: www.sawis.co.za.

St. John, B., Special to Tribune Newspapers, Chicago Tribune, Uncorked: Say 'Petite Sirah' and you've said a mouthful. Available: http://articles.chicagotribune.com/2011-03-02/features/sc-food-0225-wine-petite-sirah-20110302_1_peloursin-obscure-french-variety-petite-sirah., March 2011, 2016

Wine-searcher, www.Wine-Searcher.com, 2015, 2016

Chapter 2

Literature review

Chapter 2: Literature review

2.1 Introduction

This review provides insight into a rare variety in South Africa, Durif, also known as Petite Sirah. As of November 2014 there were 162 771 Durif vines planted on 53.67 hectares, contributing to 0.1% of the total red plantings in South Africa (SAWIS, 2015).

Petite Sirah wines are well known internationally for their dark colour and robust tannins. The wines are considered tannic and can be astringent. Because of Durif wines astringent tendency, this literature review explores tannins and perceptions of astringency in order to better understand their influence on wine mouth feel (Ribéreau-Gayon *et al.,* 2006).

Additionally, the use of sequential harvest dates as a potential method to explore different wine styles and sensory profiles is reviewed and sequential harvesting using the fruit sugar loading concept is explained. One of the goals of this research project is to determine if Durif could produce different wine profiles while using sequential harvest from grapes originating from the same site/vineyard/block.

Wines made from sequentially harvested grapes were evaluated sensorially in this research project. The review outlines the method used for sensory evaluation of the wines – the frequency of citation method.

The climatic indices used to describe the experimental plot as well as methods to measure leaf water potential are presented as both can impact on wine style.

2.2 About Durif

2.2.1 History

Dr. Francois Durif propagated Durif in 1880 at the University of Montpellier. It was a crossing (unknown at the time) between Peloursin and Syrah (Meredith *et al.,* 1999). The variety was introduced to California in 1884 by Charles McIver and referred to as Petite Sirah and planted in mixed plantings (Meredith *et al.,* 1999). In 1993, Dr. Carole Meredith confirmed the lineage of Petite Sirah. The research provided strong genetic evidence that Petite Sirah is the progeny of Peloursin (and not a clonal selection) and Syrah and that 90% of the plantings of Petite Sirah in the United States were in fact the same genetic material as Durif planted in France. In some of the commercial vineyards used in Meredith's study, it was found that some of the vineyards were inter-planted or confused in fact with Peloursin (Meredith *et al.,* 1999).

In 2002 the Bureau of Alcohol, Tobacco and Firearms (ATF) proposed that Durif be approved as a synonym for Petit Sirah based on the findings by Meredith *et al.,* However, in the same proposal

was the proposed synonymy of Zinfandel and Primitivo, which caused an outcry in the wine industry and resulted in both proposals being withdrawn in 2008 (Kelly, 2010). Final approval by the ATF to use Durif as a synonym for Petite Sirah was granted in 2010 through efforts of the *P.S I love you board* (Diaz, 2015). Other synonyms for Durif include: Dure, Druet, Plant Durif, Pinot de Romans, Pinot de l'Hermitage, Plant Fourchu, Nerin, Gros Noir, Bas Plant (Bettiga, 2003).

2.2.2 Characteristics (vineyard)

General characteristics of Petite Sirah as described by Bettiga (2003) include medium sized (150g to 225g) long conical to cylindrical compact clusters which are often winged to double. Berries are medium sized (1.7-2g), short-oval to round and are blue-black with a silvery bloom. Leaves are small to medium, deeply three-lobed, closed U-shaped petiolar sinus, short sharp teeth and the lower leaf surface is glabourous. Vines are moderately vigourous with weeping growth (Goussard, 2008). The only clone available in Napa Valley, United States, is FPS 03. Yields range from 6 to 18 tons per hectare (Bettiga, 2003). According to Bettiga (2003) Durif is a mid–late season variety and is grouped together with Cinsaut, Malbec, Nebbiolo, Tannat, Cabernet Sauvignon, and Cabernet Franc. However, this variety is not found to be as late as Cabernet Sauvignon in South Africa. Meredith *et al.,* explains that ampelographic differences between Peloursin and Durif in commercial vineyards are rarely evident. Peloursin is described to be more deeply lobed, have more convex teeth and hairless underneath compared to Durif which has a slightly tormentose leaf with sharper teeth.

Bunch rot can be a problem due to compact bunches; Durif is, however, quite tolerant of powdery mildew. Durif berries have a tendency to sunburn and shrivel. Upon maturity fruit tends to shrivel and raisin and harvest timing is important to minimize fruit weight loss (Bettiga, 2003).

2.2.3 Characteristics (wines)

Durif is well known for its dark colour and tannin, and is often used as a blending partner. It has been described as an excellent candidate for aging with blackberry, chocolate and black pepper, blueberry, liquorice and herbal sensory attributes/characteristics (Wine-Searcher, 2015). Christopher Paubert (from Stag's Leap Winery in the United States) described different Petite Sirah wines from different regions in Napa Valley as "more floral and less tannic" compared to another wine as "more spicy and concentrated".

2.3 Sugar loading concept

Sugar loading (SL) can be defined as the evolution of the quantity of sugar per berry (mg per berry) from veraison onwards (Deloire, 2011). The evolution of berry sugar accumulation could be used as a physiological indicator of the ripening process and is a practical approach when trying to obtain particular wine styles using sequential harvesting (Deloire, 2011).

Sugar evolution is described on a per berry basis, which enables the kinetics of sugar concentration to be monitored when sampling is done frequently. The calculation to determine sugar per berry is explained by Deloire (2011). The Brix of a known number of berries and berry mass is used to calculate the sugar evolution per berry per day and per gram berry. The calculation is considered an approximation due to seed volume and sugar distribution between seed and pulp (Deloire, 2011). Berry volume is considered equivalent to berry mass for these calculations (De Villiers, 1987).

The monitoring of sugar loading can be used as a diagnostic tool in vineyards as it relates to the plants physiological functioning (Deloire *et al.,* 2005). As described by Deloire (2011), sugar loading may present different profiles: rapid and continuous loading, slow or inhibited loading, loading with a plateau phase. This profiling of vineyards provides viticulturists and winemakers with more information to make decisions regarding harvest dates, irrigation needs (using the evolution of berry volume). Rapid and continuous loading is associated with vigorous growth and larger berry size. Slow rate of sugar loading and low concentration of sugar per berry is an indicator of blocked ripening or photosynthesis. This can be due to water stress, an imbalance in the source/sink relationship from excessive yields for the canopy size, mineral deficiencies or disease. Sugar accumulation profile with a plateau phase is most desirable for making consistent desired wine styles as the potential wine profile can be predetermined.

The stopping of sugar loading corresponds to maturity. The time point where sugar loading stops and accumulates less than 3 mg / berry / day is referred to as the Key Point (KP) or day zero (Figure 2.1). After the plateau phase is reached, the evolution of ripening will depend on macro- and micro-climatic factors, site (climate × soil), water availability and the canopy or leaf to fruit ratio characteristics.

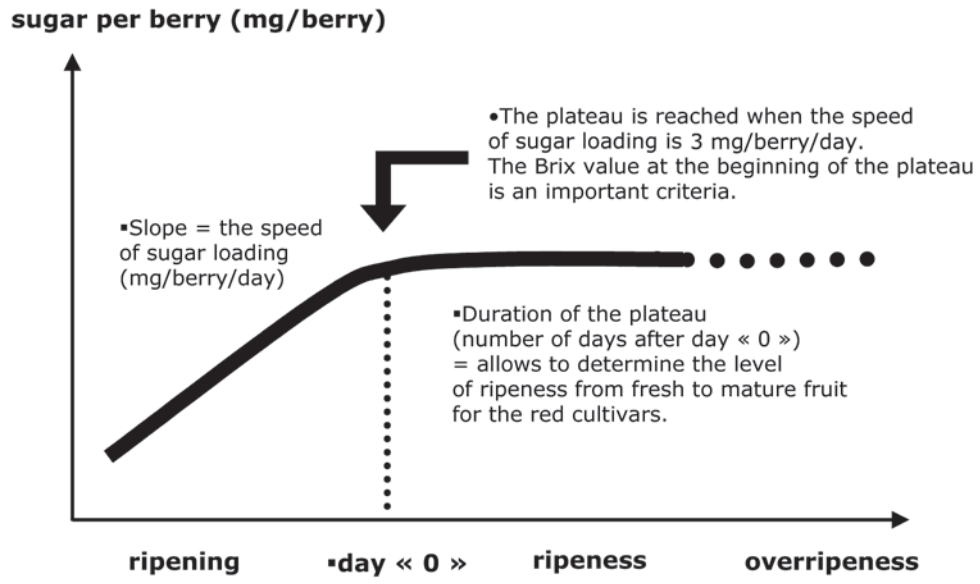

Figure 2.1: Berry sugar loading curve (Deloire 2011 in *Wynland*)

2.4 The use of sequential harvest to investigate potential wine styles

2.4.1 Choice of harvest time

There is not one optimal harvest date for a vineyard or specific site and several harvest times/periods could be adequate, but the optimization thereof is dependent on the desired wine style. The choice of harvest date is linked to grape composition (Coombe, 1992). Coombe (1992) suggested that indices based on sugar and acid concentration alone are not adequate in determining grape quality, Botes (2009) highlighted the seasonal and mesoclimatic variation when using various indices and suggests parameters (such sugar and anthocyanin concentration) should be used in combination for determining ripeness. Important criteria for harvest determination would be ease of use, reliability and ability to plan in advance.

Various methods are followed to determine potential harvest dates. For example, using one or a combination of grape analysis (Brix, pH, TA, malic acid), harvesting according to personal experience (expert knowledge) through visual observations and berry tasting — although drawbacks in training a panel are evident, as determined by Mantilla *et al.,* (2012).

2.4.2 The berry aromatic evolution

The berry aromatic evolution refers to the different wine styles that can be achieved by harvesting different grape cultivars at different dates in relation to the Key Point (Deloire, 2011; Bindon *et al.,* 2013; Nell, 2015). For Syrah, for example, these wine styles include a Fresh Fruit (FF) style reached from approximately 10 days after the KP, followed by a neutral or spicy style or pre-mature style and from approximately 20 days onwards after KP a Mature Fruit (MF) style, and later an Over Ripe style (OR) could be assessed by sensory analysis. The sensory attributes and wine mouthfeel could vary between cultivar, region, site and cultural practices, but the ripening evolution and stages are a given. For example, for Cabernet Sauvignon wines, a Fresh Fruit style can be achieved by harvesting 20 to 25 days after KP is reached and mature fruit style wines can be achieved by harvesting after 40 days (Figure 2.2). The calibration of Durif to this model has not been done and is yet to be done in South Africa.

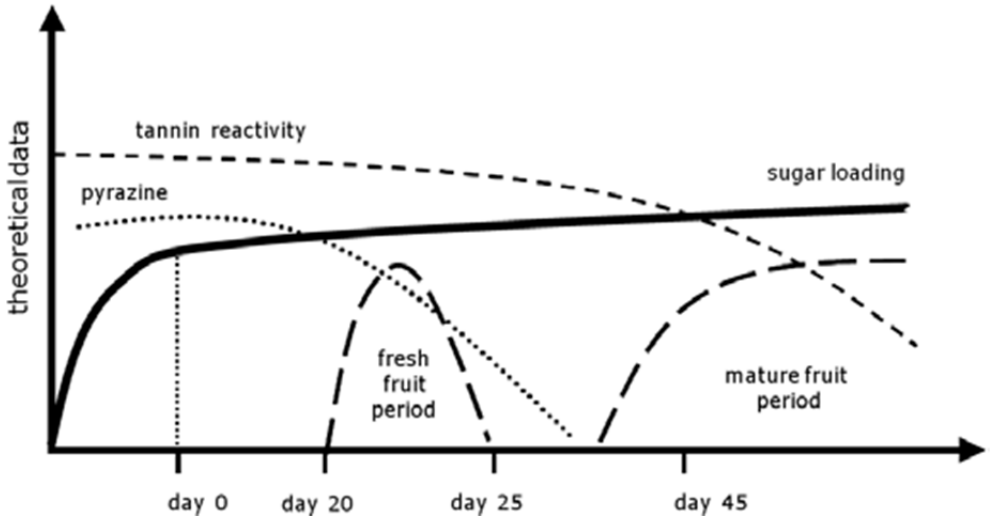

Figure 2.2: A representation of the berry aromatic evolution for Cabernet Sauvignon (Deloire 2011 in *Wynboer*)

Sugar loading is not a stand-alone solution to management of picking dates. Decisions around picking time will still include classical indicators such as Brix, pH and TA. Other indicators for determining picking date include seed coat colour, glycosyl-glucose analysis, total phenols, protein precipitation assays, grape colour measurement, anthocyanin measurement, extrability (glories method) and it is suggested by Botes (2009) to use two or more in combination. All factors need to be considered and collated with SL information to make informed decisions. For example, if the Cabernet Sauvignon pictured in Figure 2.2 is at 25 Brix at the beginning of the Fresh Fruit window it is unlikely the grapes and subsequent wine style would be favourable for a Mature Fruit profile wine due to a probable berry dehydration which will lead to an increase in sugar concentration/Brix. Abiotic factors affect, from berry set onwards, the evolution/metabolisms (accumulation or degradation) of other grape derived compounds (amino acids, nitrogen, aromatic precursors, tannin and anthocyanins, to mention a few compounds) which are important for wine composition and sensory profile, and these are not directly dependent on sugar accumulation/Brix (Jackson and Lombard, 1993).

SL can be used as an indicator of slow maturation to understand where phenolic ripeness may not reach its full potential. For example, if the rate of sugar loading is very slow or the sugar concentration seems to plateau at a low sugar concentration, this can be an indicator of vine stress and with irrigation reloading can occur. Even though sugar accumulation is not directly linked to other grape derived compounds, it is a kinetic measurement and it can be used as an indicator of the physiological state of the vines.

In white cultivars an additional indicator, berry colour evolution (Figure 2.3), can be used to determine wine style. This method uses the evolution of berry hue in conjunction with sugar loading to predict wine style. Vivelys, France, developed the technology.

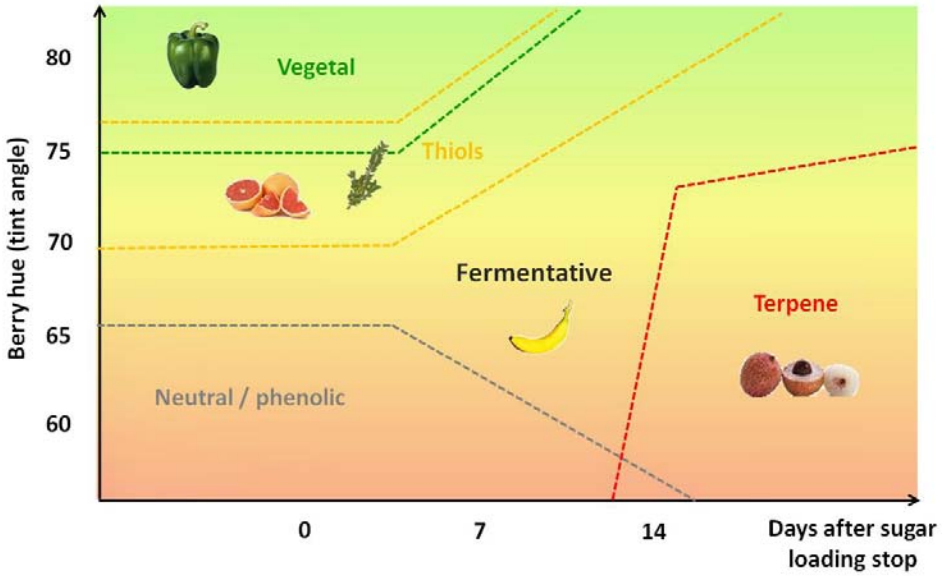

Figure 2.3: Model for berry colour evolution and wine style (Deloire 2011 in *Wynboer*)

2.4.3 Influence of harvest date on grape composition and wine style

In California, Petite Sirah is widely planted and well understood in the cellar. Michael McCay (from McCay Cellars, U.S.A) uses sequential harvesting. McCay picks in three batches with a three to three-and-a-half degree difference in Brix. The earlier pickings are described as "brighter blueberry, a softer, elegant style, brighter phenolics" and the later pickings "have a deeper, richer structure" (Boone, 2012).

Harvest date is linked to grape composition (Coombe, 1992). Linking berry composition to wine composition and in turn to wine aromatic profiles is a complex topic. Wine composition is affected by grape composition but it also depends on the winemaking techniques, the degree of extraction of several compounds present in the grapes (*e.g*. tannin and anthocyanins), yeast activity reflected in the release or partial release of non-volatile precursors (*e.g.* thiols and terpenes) and in the fermentation-related aromas(Bindon *et al.,* 2013; Antalick *et al 2015*). Sequential harvesting adds to the complexity of linking grape composition to wine profiles as differences in berry nitrogen content, sugar content and lipid status impact ester production by yeast (Bindon *et al.,* 2013; Antalick *et al 2015*). Volatile components also have interactive effects, and suppressive and synergistic effects (Sáenz-Navajas *et al.,* 2010; Antalick *et al.,* 2014, 2015) thus wine composition also has a complex relationship with wine profiles or sensory attributes.

Recent studies have shown the complex relationship between harvest date, grape composition and wine style using sequential harvest to assess wine quality (Bindon *et al.,* 2013; Deloire 2013; Šuklje *et al.,* 2014; Boss *et al.,* 2014; Šuklje *et al.,* 2014; Gil *et al.,* 2015; Cadot *et al.,* 2012; Nell 2015).

Boss *et al.,* (2014) showed that there are complex interactions between harvest timing, volatile composition of wines and Brix. Riesling grapes were sequentially harvested and were sorted

according to Brix levels for different wines. It was shown that certain positive varietal aroma compounds were positively correlated to an increase in Brix. Monoterpenes increased with an increase in Brix (up to 18 Brix) and harvest dates. Norisoprenoids were more abundant in earlier harvest dates (Boss *et al.,* 2014). The study confirms the complex relationships between wine volatile composition, harvest date and increasing Brix, and the interaction between harvest date and Brix.

Sequential harvest was used by Gil *et al.,* (2015) to investigate the effect of grape maturity and maceration length on polysaccharide composition in Cabernet Sauvignon wines. It was found that the total concentration of wine polysaccharides increase with the maceration length and grape maturity level. Yeast derived manno-proteins in wine decreased with increasing grape maturity.

In a study on Australian Shiraz from two different sites, sequential harvest was used to understand the relationships between grape composition and wine profile and aromas. Using the berry ripening evolution, fresh fruit, mature fruit and over ripe/jammy fruit style wines were made from grapes picked 12, 24 and 34 days after KP respectively. The mature fruit wines showed higher ratings for dark fruit, spicy/peppery notes and fullness/roundness. Fresh Fruit picking dates corresponded to red fruit notes, lower total soluble solids, higher acidity, α-terpinene, α-ionone, β-ionone, terpinolene, hexyl alcohols and hexyl esters in wines. Later harvest dates (mature and jammy) were correlated to dark and stewed fruit attributes, higher perception of roundness, higher total soluble solids, linalool, α- terpineol, trans-linalool oxide, guaiacol, proline and branched amino acids analysed in grape berries, dimethyl sulphide, hexyl acetate, phenyl ethyl acetate, γ-nonalactone and other esters quantified in wines (Šuklje *et al.,* 2014).

In another Australian study, the ripening-specific sensory profile of Cabernet Sauvignon of five sequentially harvested wines was investigated (Bindon *et al.,* 2014). Grape composition, wine composition and sensory analysis were assessed including a consumer preference study (Bindon *et al.,* 2014). The effect of grape composition on yeast metabolism was taken into consideration in this study. As the grapes got riper, anthocyanin, skin tannin increased; and seed tannin, total tannin, malic acid decreased (Bindon *et al.,* 2013). With an evolution of the fruit ripening the wine composition was affected as follows: total anthocyanin, total tannin, wine colour density, ester increased and there was a decrease in concentration of IBMP and C_6 volatiles (Hexanol, (Z)-3-hexen-1-ol, hexyl acetate). Wines from earlier harvests were rated higher for red fruit, red colour, fresh green attributes and rated lower for overall fruit, vanilla, fruit aftertaste, purple colour, viscosity, dark fruit, hotness, pungent and bitterness. In contrast wines from later harvests had higher ratings for dark fruit, overall fruit, hotness, pungent, opacity, bitter and earthy attributes. Positive correlations between dark fruit and dimethyl sulphide and positive correlations were found between red fruits and IBMP and C_6 alcohols were found (Bindon *et al.,* 2014).

Cadot *et al.,* (2012) investigated the effect of maceration time and harvest date on the impact of phenolic composition of wine in Cabernet Franc. It was found that with later harvested grapes, the wines had a higher tannin concentration and both tannin and anthocyanin concentrations were

effected by harvest timing. Wine from the later harvest date was associated most strongly with higher perceived colour intensity, bitterness and astringency.

2.5 Tannins and perception of astringency

Phenolic compounds are responsible for structure, mouthfeel, fullness, astringency, bitterness and roundness of wines and contribute to the quality of wine (Robichaud and Noble, 1990). It is important to note that astringency perception differs from individual to individual. Saliva is composed of proline rich proteins (PRP's), histidine-rich proteins (histatins), α-amylase and glycoproteins (lactoferrin and mucins). Salivary flow rate, viscosity, and protein composition varies between individuals and these factors have an impact on the perception of astringency (McRae and Kennedy, 2011). The salivary flow rate and composition can also be affected by the phenolic profile and the chemical and physical properties of the wine being tasted (Gawel, 1998). Higher concentrations of certain saliva proteins and a higher flow rate of saliva have been shown to generally reduce the sensation of astringency (Schwarz and Hofmann, 2008). This emphasizes the possible differences and great variation in perception of astringency between individuals.

This affects the way sensory analysis is performed and attempts have been made to develop models to simulate the way tannins react with salivary protein (causing the perception of astringency). An important property of tannin is its ability to bind with proteins (Ribereau-Gayon *et al.,* 1996). However, another problem is the difficulty in defining the type of tannins within a wine matrix for these simulation models. As well as how the tannins are bonded to salivary proteins, how other compounds effect their precipitation and whether other compounds affect the perception of astringency.

2.5.1 Phenolic composition of the berry

Phenols are secondary metabolites and are present in the skin, pulp and seed of the berry (Cheynier *et al.,* 2006). These compounds can be divided into flavonoids and non-flavonoids. Flavonoids (anthocyanins, flavan-3-ols, flavanonols and flavonols) contribute to wine colour, bitterness and astringency (Gawel, 1998). Flavan-3-ols comprise of monomers (catechins and epicatechins), oligomers and polymers (condensed tannins or proanthocyanidins). The major monomers in grapes are (+)-catechin, (-)-epicatechin and epicatechin 3-gallate. Trace amounts of (+)-gallocatechin and (-)-epigallocatechin are also found in polymers (Terrier *et al.,* 2009).

The phenolic composition of seeds consists mainly of flavan-3-ols (McRae and Kennedy, 2011). Seed tannins have a lower average degree of polymerization than skin tannins and are composed mainly of catechin and epicatechin subunits, with a greater proportion of galloylated units than skin tannins (Brossaud *et al.,* 2001; McRae and Kennedy, 2011). Flavan-3-ol monomers have been shown to be more bitter than astringent and the opposite effect was shown for flavan-3-ol polymers (Robichaud and Noble, 1990). This could explain seed tannin's contribution to bitterness in wine.

The phenolic composition of grape skin consists mainly of flavonols and anthocyanins. It has also been shown that anthocyanins produce small sensory effects on astringency and bitterness (Brossaud *et al.,* 2001). Skin tannins consist of long polymeric chains ranging from 3 to 83 flavanol subunits and contain epigallocatechin subunits, unlike seed tannin (McRae and Kennedy, 2011). The higher the degree of polymerization of tannins, the higher the capacity for binding proteins and the higher the astringency. Polymeric tannins are more astringent than the monomeric compounds catechin and gallic acid (Robichaud and Noble, 1990). The non-flavonoid compounds (phenolic acids) are the main phenol components of the pulp (Ribereau-Gayon *et al.,* 1996). However, the pulp also contains flavan-3-ols and generally has a greater molecular mass than seed tannins (McRae and Kennedy, 2011).

2.5.2 Astringency

Astringency is described as a tactile sensation and the drying-out, roughening and puckering sensation felt in the mouth (Breslin *et al.,* 1993; Gawel, 1998). Four general classes of compounds can cause astringency: polyphenols, metal salts (such as alum), acids, and dehydrating agents (such as alcohol) (Breslin *et al.,* 1993). The main astringents in grapes can be divided into three groups: flavan-3-ols, non-flavanoids, pigmented polymers (anthocyanins) (Gawel, 1998). This literature review focuses on flavan-3-ols and their polymers (condensed tannins). Polymeric flavan-3-ols and the monomeric flavan-3-ol catechin have shown both astringency and bitterness in wines (Robichaud and Noble, 1990).

The perception of astringency is a dynamic process and experiments include time intensity, total duration and maximum intensity to characterize the perception of astringency (Brossaud *et al.,* 2001). The mouthfeel wheel was developed to provide researchers and tasters with standardized terminology for describing types of astringency and mouthfeel characteristics in red wine (Gawel *et al.,* 2000). In contrast, bitterness is a taste perceived by taste receptors (Gawel *et al.,* 2000).

Mechanisms of Astringency

The exact mechanism of perceiving astringency is unclear. The most widely accepted theory according to literature is the binding and precipitation of salivary proteins and tannins resulting in decreased lubricity of saliva and increasing friction resulting in a tactile sensation (Breslin *et al.,* 1993, Boulet et al., 2016). However, there are other theories involving the viscosity of saliva, interactions with bitterness receptors, desquamation of the oral mucosa and direct interactions of astringents with the oral epithelium (Lee *et al.,* 2012). These other theories are supported by findings showing compounds with no protein binding activity having been perceived as astringent at low thresholds (Schwarz and Hofmann, 2008). This shows the perception of astringency could be related to the "free astringent compounds" and not the binding of tannin to salivary proteins (Schwarz and Hofmann, 2008). Gawel (1998) proposed that any wine component affecting the visco-elasticity of saliva could be perceived as astringent if astringency is the result of decreased lubrication.

Saliva composition

Saliva is composed of Proline Rich Proteins (PRP's), histidine-rich proteins (histatins), α-amylase and glycoproteins (lactoferrin and mucins). Salivary flow rate, viscosity, and protein composition varies between individuals and these factors have an impact on the perception of astringency (McRae and Kennedy, 2011). The salivary flow rate and composition can also be affected by the phenolic profile and the chemical and physical properties of the wine being tasted (Gawel, 1998). Higher concentrations of certain saliva proteins and a higher flow rate of saliva have been shown to generally reduce the sensation of astringency (Schwarz and Hofmann, 2008). This emphasizes the possible differences and great variation in perception of astringency between individuals.

It is also hypothesized that different types of astringents have different mechanisms of astringency and a decrease in lubricity is not essential for perceiving astringency (Lee *et al.,* 2012).

Tannin–protein binding

Tannins bind to proteins in three stages. First, tannin–protein complexes are formed by hydrophobic interactions and hydrogen bonding (Hagerman and Butler, 1978). Cross-links are then formed between the complexes forming aggregates. Finally, the precipitation of the protein-tannin complexes takes place (McRae and Kennedy, 2011). These interactions are affected by the concentration of salivary proteins and tannins as well as pH, temperature and ionic strength (McRae and Kennedy, 2011). The tannin to protein ratio and concentration affects interactions (Gawel, 1998).

2.5.3 Factors affecting tannin–protein interactions and perceived astringency

pH and acidity

Acids are part of a class of astringents. Lowering the pH has a greater effect on increasing astringency than increasing the concentration of acid, which only increases the perceived sourness (Sowalsky and Noble, 1998). The addition of tartaric acid to Merlot wine increased precipitation of proteins (Rinaldi *et al.,* 2012). This was also shown using the Saliva Precipitation Index (SPI) and sensory analysis. The wine was perceived as more astringent (Rinaldi *et al.,* 2012). However, it is not clear whether this is due to tartaric acid alone or change in pH.

Type and size of tannin

The higher the degree of polymerization of tannins, the higher the capacity for binding proteins and the higher the perceived astringency. Tannins are more astringent than the monomeric compounds catechin and gallic acid (Robichaud and Noble, 1990). Astringency intensity in wine increases with tannin concentration (Robichaud and Noble, 1990). The higher the degree of gallolylation, the higher the protein binding activity (Schwarz and Hofmann, 2008) and the higher the perceived astringency (Brossaud *et al.,* 2001).

Anthocyanins

The contribution of anthocyanins to astringency and the mechanism thereof is unclear. It is unlikely they would interact directly with salivary proteins to cause astringency as they are, like

proteins, positively charged (Gawel, 1998). Anthocyanins may have a small contribution to astringency in wine when found in combination with skin and seed tannin (Robichaud and Noble, 1990). However, pigmented polymers have reduced ability to bind and precipitate protein (McRae and Kennedy, 2011). It has also been shown that anthocyanins produce small sensory effects on astringency and bitterness (Brossaud *et al.,* 2001). In a review by Ma *et al.,* (2014), the importance of the subqualities of astringency (with reference to the terms from the Mouthfeel Wheel of Gawel *et al.,* (2000)) is highlighted and it is hypothesized that anthocyanins may have an impact on some of these mouthfeel subqualities in wine.

Ethanol

Ethanol is considered to be part of a class of dehydrating agents that are classified as astringents. Ethanol concentration has an impact on astringency perception. For example, tannins are perceived as more astringent in the presence of 13% ethanol compared to 0% ethanol (Obreque-Slíer *et al.,* 2010). Conversely, the addition of ethanol to both a model wine solution and a Merlot wine decreased the amount of salivary protein precipitated and reduced the perception of astringency indicated by a sensory panel (Rinaldi *et al.,* 2012). On treating high alcohol wines (which were described as bitter and astringent) with reverse osmosis to reduce the alcohol, Smith (2010) found that even a 0.1% difference in alcohol could be the difference between a "balanced" wine and a "hot and bitter wine". By reducing the alcohol of a 2007 Cabernet Sauvignon, the same author found "sweet spots" at 13.7% and 14.2%. On blending the wine to 13.95%, the wine was rated 25% more astringent (Smith, 2010). This demonstrates the effect of the wine matrix as well as the alcohol's effect on the perception of astringency.

Fructose, sucrose and viscosity

The addition of sucrose to wine reduces the astringency and affects the viscosity as well as the sweetness of wine (Smith *et al.,* 1996). Increasing the viscosity alone with carboxymethylcellulose decreased the perception of astringency but had no effect on bitterness perception. In contrast, by increasing the sweetness of wine with aspartame, viscosity is not affected; bitterness in wine is reduced, but not astringency. Therefore, it is hypothesised that when adding sucrose to wine, the perceived reduction in astringency is also due to the increased viscosity (Smith *et al.,* 1996). The addition of fructose to both a model wine solution and a Merlot wine decreased the amount of salivary protein precipitated and reduced the perception of astringency, as indicated by a sensory panel (Rinaldi *et al.,* 2012).

Polysaccharides and mannoproteins

Polysaccharide composition, structure and viscosity all contribute to the perception of astringency (Vidal *et al.,* 2004). The addition of commercial mannoproteins to both a model wine solution and Merlot wine decreased the amount of salivary protein precipitated and reduced the perception of astringency (Rinaldi *et al.,* 2012). Both mannoproteins and polysaccharides contribute to the "fullness" or viscosity of wine and decrease the astringency of wines. Viscosity is, however, not the only factor affecting this decrease of astringency. It could be due to the lubricating effect of polysaccharides or due to polysaccharide-tannin interactions by either increasing tannin solubility or preventing tannin-protein interactions (McRae and Kennedy, 2011). Mannoproteins may also

react differently in red wine compared to model wine due to the impact of the wine matrix on interactions (Rinaldi *et al.,* 2012). All polysaccharide families reduce the perception of astringency in model wines (Vidal *et al.,* 2004). Polysaccharides and oligosaccharides are able to modulate perceived astringency. It was proposed the ability of the carbohydrates to decrease perceived astringency was dependent on the size and tri-dimensional structure of the polysaccharide and require further study (Quijada-Morín *et al.,* 2014).

The wine matrix

The wine matrix may have a moderating effect on the astringents in the sense that a higher dosage of astringents are required to perceive the same astringency in wine compared to that of a model wine. This could be caused by high molecular weight alcohols, sugars and glycerol, all of which have an effect on wine viscosity (Goldner and Zamora, 2010).

Fruitiness

Perceived fruitiness in wine is dependent on wine composition, concentration of volatiles and synergistic effects between compounds. Fruitiness has been shown to be enhanced by low levels of dimethyl sulphide, β-damascenone, and β-ionone (Escudero *et al.,* 2007). Fruitiness has been shown to suppress vegetative characters (Hein *et al.,* 2009; King *et al.,* 2011). Sáenz-Navajas *et al.,* (2010) showed that the volatile composition influences the taste perception in white wines and less so in reds. Astringency and bitterness of red wine is related to the non-volatile matrix composition. Fruity aroma is inversely correlated to astringency and bitterness (Sáenz-Navajas *et al.,* 2010). Cliff *et al.,* (2007) observed a masking of wine fruitiness when adding grape seed extract (condensed tannin with a lower degree of polymerization than skin tannin) to wines and an increase of the perceived astringency. In addition to the increase in perceived astringency, an increase in the woody/earthy aroma and increase in colour were observed.

2.6 Frequency of citation method

The frequency of citation method is one of several sensory evaluation methods (descriptive analysis, sorting, free description, *etc.*) available for cultivar characterization and has been used in several recent studies (Campo *et al.,* 2010, Campo *et al.,* 2008, Sáenz-Navajas *et al.,* 2010). Frequency of citation method requires panelists to select a specific number of descriptors per wine from a pre-established (and trained) reference list. The descriptors, which are commonly cited by panelists, are used for statistical analysis. This method was used to discriminate between aroma profiles of neutral Spanish varietals (Campo *et al.,* 2008) and it was found there were no significant differences between the wines other than Verdejo and Sauvignon Blanc.

In a study on Burgundy Pinot Noirs, a comparison between traditional descriptive analysis and the frequency citation method was conducted to investigate the differences and similarities in sensory maps, panel monitoring and the practical aspects (Campo *et al.,* 2010). Campo *et al.,* (2010) showed that an advantage is that the panel can be used to evaluate other wines after a few training sessions, as their knowledge base of general descriptors is large. The frequency citation

method provides a more complete image compared to descriptive analysis as the descriptor list is not reduced.

In a study using reconstituted Chardonnay and Tempranillo wines the frequency citation method was used to show the effects of the wine volatiles on the perception of taste and astringency (Sáenz-Navajas *et al.,* 2010). In white wines the addition of fruity volatile extracts decreased astringency and bitterness. In red wine there were no significant changes in taste and astringency, suggesting that the non-volatile fraction in these wines drive mouthfeel and astringency.

2.7 Conclusion

Durif is a specific variety with intense colour and notable astringency. As a stand-alone wine or blending component it has a lot of potential to offer South African red wine. Little is known about Durif in South Africa and further studies using this grape variety will benefit winemakers and encourage more plantings of this variety. Wine profiles have not been explored for Durif in South Africa and further study on this topic is necessary to understand what Durif can offer and to describe the potential fruit ripening evolution. Choice of harvest timing is key. Sequential harvesting is a method which can be used to better define the optimum ripeness for a specific wine style. It has been shown how, in previous studies, harvest-timing impacts on grape composition, wine composition, style and sensory perceptions. Further study to identify key indicators and patterns of precursors in grapes can help further understand the ripening-specific sensory profile of wines or varietal groups.

2.8 Bibliography

Antalick, G., Šuklje, K., Blackman, J.W., Meeks, C., Deloire, A. and Schmidtke, L.M., 2015. Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry*.

Antalick, G., Perello, M. and De Revel, G., 2014. Esters in Wines: New Insight through the Establishment of a Database of French Wines. *American Journal of Enology and Viticulture*, 65(3), pp. 293-304.

Bettiga, L.J., 2003. *Wine grape varieties in California.* Oakland, California: University of California, Agriculture and Natural Resources.

Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M. and Francis, I.L., 2014. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry,* 154(0), pp. 90-101.

Bindon, K., Varela, C., Kennedy, J., Holt, H. and Herderich, M., 2013. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry,* 138(2–3), pp. 1696-1705.

Boone, V., Wine Enthusiast, Taming Petite Sirah. Available: http://www.winemag.com/Web-2012/Taming-Petite-Sirah/ , 2012, 2016 (last update).

Boss, P.K., Böttcher, C. and Davies, C., 2014. Various Influences of Harvest Date and Fruit Sugar Content on Different Wine Flavor and Aroma Compounds. *American Journal of Enology and Viticulture,* 65(3), pp. 341-353.

Botes, M.P., 2009. *Evaluation of parameters to determine optimum ripeness in Cabernet Sauvignon grapes in relation to wine quality*. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Boulet, Jean-Claude, et al., 2016 "Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency." *Food chemistry* 190 (2016): 357-363.

Breslin, P.A.S., Gilmore, M.M., Beauchamp, G.K. and Green, B.G., 1993. Psychophysical evidence that oral astringency is a tactile sensation. *Chemical senses,* 18(4), pp. 405-417.

Brossaud, F., Cheynier, V. and Noble, A.C., 2001. Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research,* 7(1), pp. 33-39.

Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V., 2012. Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. *Analytica Chimica Acta,* 732, pp. 91-99.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference,* 21(1), pp. 44-55.

Campo, E., Do, B.V., Ferreira, V. and Valentin, D., 2008. Aroma properties of young Spanish monovarietal white wines: a study using sorting task, list of terms and frequency of citation. *Australian Journal of Grape and Wine Research,* 14(2), pp. 104-115.

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture*, *57*(3), 298-305.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. *American journal of Enology and Viticulture.,* 43(1), pp. 101.

De Villiers, F.S., 1987. *n Vergelykende ampelografiese en ampelometriese studie van die tros van verskillende wyndruifcultivars,* .

Deloire, A., Kraeva, E., Martin, M. and Hunter, J., 2005. Sugar loading and phenolic accumulation as affected by ripeness of Syrah/R99 grapes. *XIV International GESCO Viticulture Congress, Geisenheim, Germany, 23-27 August, 2005.* 2005, Groupe d'Etude des Systemes de Conduite de la vigne (GESCO), pp. 574-580.

Deloire, A., 2013. Wine aroma and grapevine berry ripening: How to capture the complexity. *Winetitles*

Deloire, A., 2011. The concept of berry sugar loading. *Wynboer,* 257, pp. 93-95.

Deloire, A. and HEYNS, D., 2011. The leaf water potentials: Principles, method and thresholds. *Wineland,* 265, pp. 119-121.

Diaz, J., 2015-last update, P.S I love you. Available: http://www.psiloveyou.org.

Escudero A, Campo E, Fariña L, Cacho J,Ferreira V, 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry,* 55(11), pp. 4501-10.

Ferrer-Gallego, R., García-Marino, M., Miguel Hernández-Hierro, J., Rivas-Gonzalo, J.C. and Teresa Escribano-Bailón, M., 2010. Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Analytica Chimica Acta,* 660(1–2), pp. 22-28.

Gawel, R., 1998. Red wine astringency: a review. *Australian Journal of Grape and Wine Research,* 4(2), pp. 74-95.

Gawel, R., Oberholster, A. and Francis, I.L., 2000. A 'Mouth-feel Wheel': terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research,* 6(3), pp. 203-207.

Gil, M., Quirós, M., Fort, F., Morales, P., Gonzalez, R., Canals, J. and Zamora, F., 2015. Influence of Grape Maturity and Maceration Length on Polysaccharide Composition of Cabernet Sauvignon Red Wines. *American Journal of Enology and Viticulture,* .

Goldner, M.C. and Zamora, M.C., 2010. Effect of polyphenol concentrations on astringency perception and its correlation with gelatin index of red wine. *Journal of Sensory Studies,* 25(5), pp. 761-777.

Goussard, P. G. (2008). *Grape cultivars for wine production in South Africa*. Cheviot Publishing.

Hagerman, A.E. and Butler, L.G., 1978 The specificity of proanthocyanidin-protein interactions. *The Journal of biological chemistry,* 256(9), pp. 4494.

Huglin, P., 1978. Presentation internationale uniforme des resultats d'essais viticoles [teneur en sucre des mouts, acidite totale, rendement au vignoble, donnees bioclimatiques]. *Bulletin de l'OIV,* .

Jackson, D. I., Lombard,P.B., 1993. Environmental and management practices affecting grape composition and wine quality. *American journal of Enology and Viticulture.,* 44(4), pp. 409-430.

Kelly, R., 2010. *The Science of Wine Varietal Labeling Regulations: The Cases of Zinfandel and Petite Sirah.*

Lee, C.A., Ismail, B. and Vickers, Z.M., 2012. The Role of Salivary Proteins in the Mechanism of Astringency. *Journal of Food Science,* 77(4), pp. C381-C387.

Mcrae, J.M. and Kennedy, J., 2011. *Wine and Grape Tannin Interactions with Salivary Proteins andTheir Impact on Astringency: A Review of Current Research.*

Meredith, C.P., Bowers, J.E., Riaz, S., Handley, V., Bandman, E.B. and Dangl, G.S., 1999. The Identity and Parentage of the Variety Known in California as Petite Sirah. *American Journal of Enology and Viticulture,* 50(3), pp. 236-242.

Nell, M., 2015. *Sensory characterisation of several red cultivar (Vitis vinifera L.) wines, using berry sugar accumulation as a physiological indicator and sequential harvest*.

Obreque-Slíer, E., Peña-Neira, A. and López-Solís, R., 2010 Enhancement of both salivary protein-enological tannin interactions and astringency perception by ethanol. *Journal of Agricultural and Food Chemistry,* 58(6), pp. 3729.

Olarte Mantilla, S.M., Collins, C., Iland, P.G., Johnson, T.E. and Bastian, S.E.P., 2012. Review: Berry sensory assessment: concepts and practices for assessing winegrapes sensory attributes. *Australian Journal of Grape and Wine Research,* 18(3), pp. 245-255.

Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J.C., Doco, T. and Escribano-Bailón, M.T., 2014. Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry,* 154(0), pp. 44-51.

Ribereau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D., 2996. Phenolic Compounds. *Handbook of Enology Volume 2, The Chemistry of Wine, Stabilizationa dn Treatments.* second ed. England: John Wiley and Sons Ltd, pp. 142-203.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B. and Lonvaud, A., 2006. *Handbook of Enology, The microbiology of wine and vinifications.* John Wiley & Sons.

Rinaldi, A., Gambuti, A. and Moio, L., 2012. Precipitation of Salivary Proteins After the Interaction with Wine: The Effect of Ethanol, pH, Fructose, and Mannoproteins. *Journal of Food Science,* 77(4), pp. C485-C490.

Robichaud, J.L. and Noble, A.C., 1990. Astringency and Bitterness of Selected Phenolics in Wine. *Journal of Science Food Agric,* 53, pp. 343-353.

Sáenz-Navajas, M., Campo, E., Fernández-Zurbano, P., Valentin, D. and FERREIRA, V., 2010. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry,* 121(4), pp. 1139-1149.

Sawis, SAWIS. Available: www.sawis.co.za., 2015, 2016

Schwarz, B. and Hofmann, T., 2008. Is there a direct relationship between oral astringency and human salivary protein binding. *European food research and technology = Zeitschrift fur Lebensmittel-Untersuchung und -Forschung.A,* 227(6), pp. 1693.

Smith, A.K., June, H. and Noble, A.C., 1996. Effects of viscosity on the bitterness and astringency of grape seed tannin. *Food Quality and Preference,* 7(3–4), pp. 161-166.

Smith, C., 2010. Astringency and harmony in tannins.(Postmodern Winemaking). *Wines & Vines,* 91(9), pp. 57.

Sowalsky, R.A. and Noble, A.C., 1998. Comparison of the effects of concentration, pH and anion species on astringecy and sourness of organic acids. *Chemical senses,* 23, pp. 343-349.

St. John, B., Special to Tribune Newspapers, Chicago Tribune, Uncorked: Say 'Petite Sirah' and you've said a mouthful. Available: http://articles.chicagotribune.com/2011-03-02/features/sc-food-0225-wine-petite-sirah-20110302_1_peloursin-obscure-french-variety-petite-sirah., March 2011, 2016

Šuklje, K., Deloire, A. and Lisjak, K., 2014. *Sauvignon blanc aromatic profile: The sweet spot from a vineyard perspective.*

Šuklje, K., Antalick, G., Meeks, C., Blackman, J.W., Deloire, A. and Schmidtke, L.M., November 2014. Optimising harvest date through use of an integrated compositional and sensory model: a proposed approach for better understanding of ripening of autochthonous varieties? *2nd International Oneoviti symposium* November 2014.

Terrier, N., Ollé, D., Verriès, C. and Cheynier, V., 2009. *Biochemical & Molecular Aspects of Flavan-3-OL Synthesis During Berry Development.* Springer Netherlands.

Tonietto, J. and Carbonneau, A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology,* 124(1), pp. 81-97.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V. and Waters, E., 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry,* 85(4), pp. 519-525.

Wine-searcher, www.Wine-Searcher.com, 2015, 2016

Chapter 3

2012 vintage – A diagnostic year

Chapter 3: 2012 vintage – A diagnostic year

3.1 Introduction

The first year of experimentation (2012) was used to improve the 2013 layout and experimentation, and redefine some research questions and goals. It was the first time Durif had been sequentially harvested at Fairview Winery and picking the fruit for the first harvest with such a low Brix (22.05°B) was considered bold by the winemakers. The irrigation scheduling was closely followed with several PLWP and SWP readings throughout the season to ensure the vineyard did not suffer from water stress. Sugar loading (berry sugar accumulation) was used to propose picking times for a fresh fruit (FF) and mature fruit (MF) style of wine. The exact picking dates for FF and MF style wines were unknown at the time and the Merlot/Shiraz ripening models (Deloire, 2011; 2013) were used as a guideline. Wines were made from each proposed harvest window and assessed using the frequency of citation method. The aromatic attributes (or most highly cited attributes) of Durif in South Africa were reported and the mouthfeel was assessed for the first time in relation to sequential harvesting.

The main drawback for the 2012 experimentation was the specific part of the selected block. The experimental plot was selected in September 2011 and at the time it appeared fairly homogenous. However, later in the season (post-*véraison*) the block showed heterogeneity down the slope. The vines at the bottom of the slope appeared more vigorous compared to the vines at the top of the slope. It was for this reason that the plot layout was divided into a low vigour (LV) and high vigour (HV) section in early February 2012, just before harvesting. This resulted in the fragmentation of the plot. The harvest timing for the two vigours was different, as the berry sugar loading patterns for the high vigour and low vigour sections were different. The low vigour vines had two additional harvest dates which resulted in four LV harvest stages compared to two HV harvest stages.

There was a difference in summer canopy management of the experimental plot compared to the rest of the block, which was used commercially. After berry set (Eichhorn Lorenz stage 29) the lateral shoots (only in the bunch zone) were removed, exposing the bunches in the experimental plot compared to the commercial plot where 20% leaf removal (Eichhorn Lorenz stage 29) in the bunch zone on the morning-sun side of the canopy was carried out. The commercial part of the block was also used for experimentation in 2012 and a high vigour and a low vigour section was used. As a result of this complex layout, this vintage was used to build a knowledge base for the 2013 vintage. The data has been presented in the best possible way and in order to use repeats, it meant the opportunity to compare vigours and treatments was lost in some places. The winemaking was also not done in replicate, which should be taken into consideration. The 2012 vintage was used to assess the block and find the most homogenous section for the 2013 experimentation. The findings of 2012 were used to improve the plot layout and harvest timing in 2013: it was the first year that calibration of the berry sugar accumulation model for Durif using sequential harvesting was applied.

3.2 Materials and methods

3.2.1 Climate and climatic indices

A weather station, based 150 meters from the plot, was used to measure rainfall, daily maximum and minimum temperature, average hourly wind speed, and total hours with wind speed greater than 5 m/s. In addition, the following were also measured: daily maximum and minimum humidity, total rainfall, total radiation, total reference evapotranspiration (calculated according to the FAO 56 Formula; mm/day), and water consumption value (mm/day, ET0 values multiplied with Crop Factor). The weather station data was used to calculate Winkler Index, FNI and HI indices (Tonietto and Carbonneau, 2004).

A mesoclimatic data logger (TinyTagTM, United Kingdom) positioned in the experimental vineyard was used to measure temperature and humidity every 15 minutes during the growing season (in housing above the canopy). The weather station data and data logger (TinyTagTM, United Kingdom) were used to calculate Winkler index, FNI and HI indices (Tonietto and Carbonneau, 2004). These results are presented together with 2013 climatic data in Chapter 4.

3.2.2 Experimental plot layout

The experimental vineyard (33°46'1.58"S, 18°55'25.11"E) is part of an existing 1.16ha Durif / Petite Sirah vineyard on Fairview farm, on the southern slope of Paarl mountain in the Paarl wine region, South Africa. The vineyard *Vitis vinifera* cv.Durif (clone DF1A) on 101-14 Mgt rootstock was planted in 2006. The vineyard was selected because it is one of the oldest blocks of Durif at Fairview farm and historically produces good quality wine. The vineyard is small enough to keep it separate for experimental use. The soil type is Oakleaf. The vineyard is trellised on a 5 vertical shoot positioning (VSP) wire perold trellis system and has supplementary drip irrigation (2.3 L/hr). Vine spacing is 1.5 x 2 m. The vineyard is planted on a gentle slope with NW/SE row direction.

The Durif used for 2012 was the same block as the 2013 study. However, a different part of the block was used. The experimental plot consisted of a High Vigour (HV) zone and a Low Vigour (LV) zone. Each zone consisted of four panels (five vines per panel) across six rows. Two buffer panels separated the two vigour zones. The layout was confirmed by hyperspectral imaging post-véraison (January 2012). The remaining part of the block, which was commercially farmed by Fairview Winery, was used as a comparison. The vineyard area (commercial section) used in the study was also divided into a Low Vigour (FV LV) and a High Vigour (FV HV) zone for sampling. This area was two rows apart from the experimental vineyard and was also four panels by six rows wide. The main differences between the commercial vineyard and experimental vineyard in 2012 were the summer canopy management practices. The experimental plot had lateral shoot removal at pea size and the commercial plot had leaf removal on the morning sun side.

Figure 3.1: Plot layout (33°46'1.58"S, 18°55'25.11"E) with (1) FV LV, (2) FV HV, (3) LV, (4) HV

3.2.3 Vineyard management during 2011/2012 growing season

The vineyard was pruned to two bud spurs (9 August 2011). The vineyard was suckered (Eichhorn Lorenz stage 12-15) on 30 September 2011 and an additional suckering was done on 2 November 2011 (Eichhorn-Lorenz stage 19-21) when the shoots were positioned vertically and wires lifted. In the experimental plot two lateral shoots were removed from the bunch zone (Figure 3.2 and Figure 3.3) after set (30 November 2011) and a tipping action was conducted at the same time. Lateral shoots in the bunch zone were removed to expose the bunches to sufficient sunlight. This canopy management practice was favoured above leaf removal as the height of the bunch zone varied through the canopy. In the commercial section of the vineyard strong leaf removal (25%) was done on the morning sun side of the canopy between the cordon and the bunch zone. This is preferred for commercial grape/wine production because the cost of labour is considerably less at twenty man-hours per hectare (Rand 0.13 per vine) compared to sixty for lateral shoot removal (Rand 0.22 per vine) (Fairview 2012).

Figure 3.2: Distribution of light in the canopy after lateral shoot removal (3 December 2011)

Figure 3.3: Lateral shoot removal plot done post véraison

3.2.4 Monitoring grapevine water status

Grapevine water status was monitored using a pressure chamber according to the technique described by (Scholander *et al*., 1965). Pre-dawn Leaf Water Potential (PLWP, ψ_{plwp}) and Stem Water Potential (SWP) were measured in the 2012 and 2013 seasons. Additional information available for decision-making regarding irrigation scheduling included: evapotranspiration values, soil water probes (*DFM* data loggers) and Neutron probes, which were in use commercially on

Fairview farm. In 2012 the irrigation programme at Fairview farm was calibrated using SWP and PLWP, evapotranspiration values and soil water probes.

The PLWP was done from véraison weekly before and after irrigation (3 readings per panel and 20 panels were measured per date) in order to calibrate the irrigation management programme. SWP was measured once per week (1 reading per panel and twenty panels were measured per date) from November 2011. The aim was to manage the irrigation to ensure that during the growing season PLWP was higher than -400 KPa and SWP was greater than -1000 KPa (mild–moderate water deficit). Post-véraison and closer to harvest, irrigation was used to maintain PLWP higher than -600 KPa and ensure SWP was greater than -1200 KPa. The calibration of the irrigation in 2012 was used to manage the irrigation in 2013.

3.2.5 Normalized difference vegetation index (NDVI)

The Normalized Difference Vegetation index (NDVI) is used to classify vigour of vegetation (Lloyd, 1990). Outline Imagery™ took Hyperspectral images of the plot. Data was projected on Universal Transverse Mercator using WGS 84 datum. Resolution used was 0.25m. The colour image band configuration used was as follows; Band 1: Red (650 to 680nm with peak at 664.67nm), Band 2: Green (550 to 580nm with peak at 568.42nm), Band 3: Blue (450 to 480nm with peak at 465.88nm). The false colour infrared band configuration used was as follows; Band 1: NIR (850 to 900nm with peak at 870.53nm), Band 2: Red (650 to 680nm with peak at 664.67nm), Band 3: Green (550 to 580nm with peak at 568.42nm). These images were used to confirm the LV and HV sections of the plot and were later used to determine a better position for the 2013 plot.

3.2.6 Monitoring the tempo of ripening

Berry sampling

Sampling was always done in the morning before 10:00 to avoid the influence of the daily transpirational flux. Sampling between 10 January 2012 and 6 February 2012 was done per panel (five consecutive vines between two poles): 10 berries were sampled randomly across the panel and this was repeated 3 times for each panel. Four panels were sampled across 3 rows. On processing the data, it was found that there were vigour differences in the block. Based on SWP and PLWP and visual cues, the experimental plot was divided into two sections: low vigour (LV) and high vigour (HV). From 6 February 2012 (Eichorn-Lorenz stage 36-37) the samples were drawn across four panels for each vigour type. LV sampled from panels numbered 5 to 8 and HV from panels 11 to 14, leaving two buffer panels between the sections. 100 berries with two repeats were sampled randomly from bunches for each treatment.

Sampling in the commercial section of the vineyard commenced late January 2012 and the sampling took place in the same panels (LV 5-8 and HV 11-14) across six rows in the leaf removal section of the block. 100 berries with two repeats were sampled randomly from bunches for each treatment.

Berries were cut at the pedicel. Part of the samples were used for berry fresh mass and Brix, and the remaining fruits (minimum of 50 berries) were frozen at -80°C for biochemical analyses.

3.2.7 Sequential harvest

Determining harvest date

In 2012 the sugar loading curve was determined for each part of the vineyard: high vigour, low vigour and FV high vigor and FV low vigour as each section showed differences in sugar accumulation per berry. Harvest dates were predetermined according to the berry sugar loading model for each section in the plot: high vigour (HV), low vigour (LV) and commercial high vigour (FV HV) and commercial low vigour (FV LV). Two harvest dates were chosen according to the model for Merlot, Cabernet Sauvignon and Shiraz (Deloire, 2011; Deloire, 2013) for both the low vigour plots and four harvest dates were chosen for both the high vigour plots.

Table 1: 2012 harvest dates for the commercial plots (FV HV and FV LV) and experimental plots (HV and LV)

Commercial plot		Experimental plot	
FV HV	FV LV	HV	LV
	17/02/2012		21/02/2012
27/02/2012	27/02/2012	27/02/2012	27/02/2012
	02/03/2012		05/03/2012
09/03/2012	09/03/2012	09/03/2012	09/03/2012

Harvesting and winemaking

For each treatment only one vine per panel was selected per harvest date (grapes were not harvested from every panel). The entire vine was harvested to avoid altering the physiological functioning of the berries by crop thinning and to reduce the risk of sampling or harvesting from this vine that has had its crop thinned.

Grapes were delivered to the experimental cellar of the University of Stellenbosch, South Africa. The grapes were crushed and destemmed into 50 L round plastic containers and 50 mg/L SO_2 was added. The must of each batch was analysed for pH, TA and Brix. The grapes were co-inoculated with D21 yeast (30 g/hL, Lallemand), which was rehydrated with 30 g/hL *Go ferm protect* (Lallemand), and 0.01 g/L *Enoferm Alpha* (Lallemand) that was added 24 hours later. Fermentation took place at 25 °C with punch downs (*pigeage)* 3 times daily. *Fermaid K* (Lallemand) was added at a dose of 25 g/hL after 5°B had fermented. After approximately 5 days the wine reached -1°B, the wine was racked, the skins were pressed (to 1 Bar) and the free run and press wine were combined and cooled to 20 °C to complete malolactic fermentation. On completion of malolactic fermentation, the wine was racked off the lees, 50 mg/L SO_2 was added and the wine was cold stabilized (3 weeks at -4 °C). The free sulphur was adjusted before bottling to 40 mg/L. Wines were bottled under screwcap. Wines were allocated a batch code number that was used in some of the statistical analysis.

3.2.8 Determining grape composition

Sugar and organic acid analysis

The frozen berries were allowed to thaw to room temperature (20-22 °C). 10 berries were weighed in 50 mL tubes and homogenized. Deionized water was added to facilitate homogenization and after rinsing the homogenizer the final volume of pulp and water was 30 mL. The pulp was ultrasonicated (Branson 5510, United States) at room temperature for 20 minutes and centrifuged (Hermle Labortechnik GmbH Z366) at 10 000 rpm until the supernatant was clear. The supernatant was collected and stored at -4 °C prior to analysis using Arena 20XT (Thermo Electron Oy, Finland). The analysis included glucose, fructose, malic acid and tartaric acid, and were done by enzymatic reactions. Samples were drawn on 12 dates and 3 biological repeats were tested for each date.

Anthocyanin extraction from whole berries and measuring total phenolics

The frozen berries were allowed to thaw to room temperature (20-22 °C). 40 berries were weighed in a tube and homogenized. Extraction solvent (Methanol/water (70/30) adjusted to pH 1.5 with HCl, 10 mL) was added for each gram of berry and the mix was ultrasonicated for 45 min and the tube was shaken every 15 min. The solution was centrifuged at 4500 rpm for 10 minutes, the supernatant was collected in test tubes, diluted 10x with dilution solvent (1 M HCl), vortexed for 30 seconds and allowed to stand for 1 hour. The extract was transferred to a 1mm quartz cuvette and the absorbance was read at 520nm (total anthocyanins) and 280nm (total phenolics). The analysis was performed on all the samples (18 dates; 3 repeats) and 2 technical repeats were performed.

3.2.9 Determining wine composition

Analyses including pH, TA, alcohol, and residual sugar were performed. Tannins (BSA precipitation method (Hagerman and Butler, 1978)), total red pigment colour, wine colour density, wine colour hue and degree of red pigment colouration (South African Wine Laboratories Association (SAWLA) 2002) were determined.

Red wine colour and total anthocyanins

The wine pH was determined and absorbance was measured in duplicate using a 1 mm quartz cuvette at 420, 520 and 620 nm (A_{420}, A_{520}, A_{620}) (Specord50, Analytikjena, Germany). A second reading was done at low pH by adding 5 mL 1N HCl to 100µl wine, mixing and allowing it to stand for 3 hours before reading the absorbance at 280 nm and 520 nm. 620 nm was included to include the blue component of young red wines. The results were multiplied by 10 to compensate for the 1 mm cuvette used.

The following values were determined:

- Total red pigment = A^{HCl}_{520}
- Wine colour density/intensity (at actual wine pH and SO_2 level) = $A_{420} + A_{520} + A_{620}$

- Wine colour hue (at actual wine pH and SO_2 level) = A_{420} / A_{520}
- Degree of red pigment colouration (at actual wine pH and SO_2 level) = (A_{520} / A^{HCl}_{520}) x 100%.
- Total Phenols = A^{HCl}_{280}

BSA precipitation method for tannin analysis

The following solutions were used:

- Buffer A: 6 mL glacial acetic acid and 5 g NaCl to 500mL distilled water with the pH adjusted to 4.9.
- Buffer B: 2.5 g KH2T + 60 mL EtOH to 500 mL distilled water with the pH adjusted to 3.3.
- Buffer C: 25 mL triethanolamine + 25 mg SDS to 500 mL distilled water with the pH adjusted to 9.4. $FeCl_3$ solution: 0.27 g $FeCl_3.6H_20$ + 98 µL 32% HCL to 100 mL with distilled water. The solution should be protected from light and stored in the refrigerator.
- BSA solution: 1 mg/l Bovine serum albumin dissolved in Buffer A.

The procedure follows: 500 µL of wine was added to 1 mL BSA solution, vortexed and allowed to stand for 15 minutes before centrifuging (Eppendorf 5415D) for 5 minutes at 14 000 rpm. The supernatant was removed and the pellet was rinsed twice with 1 mL of Buffer A. 250 µL Buffer A was added to the pellet and centrifuged for 1 minute at 14 000 rpm. The supernatant was removed. 875 µL Buffer C was added to the supernatant and vortexed until the pellet was dissolved and allowed to stand for 10 minutes. The spectrophotometer (Specord50, Analytikjena) was zeroed with 875 µL Buffer C. Samples were read at 510 nm in disposable cuvettes and then 125 µL $FeCl_3$ was added to each sample including the reference sample and vortexed. After 10 minutes the reference sample was used to zero the spectrophotometer and the final readings were done.

A standard curve was done in triplicate with varying concentrations (50–500 µl catechin solution and 825–375 µl Buffer C) of catechin solution (0.1 g catechin dissolved in 10 mL EtOH diluted to 100 mL with distilled water). 125 µL $FeCl_3$ was added to each sample of catechin and Buffer C including a reference sample of 875 µL Buffer C and vortexed. After 10 minutes the reference sample was used to zero the spectrophotometer and the readings were done at 510 nm. The repeats were averaged and a catechin standard curve was used to convert the absorbance values to catechin units (mg/L).

3.2.10 Sensory analysis

The frequency of citation method (Campo *et al.,* 2010) was used for odour description and intensity scores were used for mouthfeel ratings. A panel of 42 members was recruited. The panel was not paid, and the training sessions were presented as a wine tasting course. The panel consisted of 42 people, of which 37 completed all the evaluations and training and 34 were used

for sensorial analysis. The objective was to describe the attributes of the berry aromatic sequences from different stages of harvesting.

Panel training

The training consisted of two phases: general training and cultivar specific training. The general training of the panel was 1 hour once per week for 11 sessions over a period of four months. A list of terms (Appendix B.1) was used for training and the panelists were provided with various reference standards representative of the list of aromas, as well as mouthfeel perceptions. Standards (Appendix B.1) were given to the panelists blind and they were asked to identify the aroma. Aromas were presented to panelists in themes as described by Nell (2015), for example, all floral aromas were assessed in one training session. The list of 101 terms (Campo *et al.* 2008) used to describe the wines was reduced and grouped during the training sessions. Various wines (red and white) were used for the general training, particularly ones with obvious aroma or mouthfeel perceptions. The panelists described the aromas of the wines and also gave intensity ratings for sweetness, sourness, astringency and bitterness on a six-point scale. The taste standards were prepared as follows:

- Bitter – 10g/l Urea
- Sour – 0.8g/l Tartaric acid
- Sweet – 8 g/l Fructose
- Astringency – 2g/l Alum de K
- Astringency and bitter – 2g/l Alum de K and 10 g/l Urea

The specific training consisted of three sessions. These sessions allowed panelists to become familiar with Durif wines. During the training, two wines were evaluated in replicate and the judge's reproducibility index was calculated. The Durif evaluated for the cultivar-specific training consisted of three commercial samples, Charlie Herring (2012 Stellenbosch Durif), Spotswood (2011 Stellenbosch Durif), and Fairview (2009 Paarl Durif), two experimental wines from the research project, and three barrel samples from Fairview Winery. During the cultivar-specific training sessions, alcohol perception was added to the list of mouthfeel. Aromatic descriptors identified by at least three panelists were kept on the descriptors list. This final evaluation list consisted of 60 terms arranged according to 8 families and their subfamilies (Appendix B.1).

Wine evaluation

The wines were poured into *Institut National des Appellations d'Origine* (INAO) approved glasses, 25 mL per glass and covered with a petri dish and arranged randomly. Panelists were asked to smell each sample and choose a maximum of 5 descriptors per wine (see Appendix B for tasting sheet). The samples were then tasted to evaluate the intensity of sweetness, sourness, bitterness, and astringency on a six-point scale. Panelists were asked to spit the wines and to take a sip of water between each sample.

Analysis of panel performance

The reproducibility index (R_i) is calculated for each panelist to assess their performance in evaluating replicate samples (Campo *et al.* 2008). R_i is calculated as follows:

$$R_i = \Sigma[2 \times des_{com}/(des_{rep1} + des_{rep2})]/n$$

Where des_{com} is the number of common terms used by the panelist in the replicates, des_{rep1} the number of common terms in replicate 1 and des_{rep2} for replicate 2, n is the number of replicated wines. The R_i values of panelists with values equal to or less than 0.2 were excluded (Campo *et al.* 2010). The global panel reproducibility was explored by submitting a contingency table of the most cited terms (citation frequency of at least 15%) to correspondence analysis. For panel performance for mouthfeel (as for traditional descriptive analysis), a three-way Analysis Of Variance (ANOVA) was performed on each attribute.

Wine aroma

A contingency table was created with wines and their descriptors and the sum of the citation frequencies of each. Only panelists with a high enough R_i were used. Only descriptors cited by at least 25% of the panel (for one wine) were used. Where replicates were used, the citation frequencies were averaged. The terms were ranked according to citation frequency for each wine to determine the most relevant attributes for each wine. The contingency table was submitted to correspondence analysis to determine the relationship between wine and descriptors. A Hierarchical Cluster Analysis (HCA) was applied to the factorial coordinates of the wines in the spaces to identify groups of wines with similar characteristics.

Wine mouthfeel

Principal Component Analysis (PCA) was performed on the attributes that showed significant differences. An HCA was applied to the factorial coordinates of the wines in the spaces defined by PCA.

3.2.11 Statistical analysis

Data was subjected to analysis of variance (ANOVA). Mean comparisons were performed using Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$). Mean comparisons with confidence intervals set at 0.95 were used to show trends (Software: Statistica).

3.3 Results and discussion

3.3.1 Climatic indices and monitoring grapevine water status

The results of the climatic indices are presented in Chapter 4 with the 2013 results. The results of SWP and PLWP show that irrigation was managed effectively as the SWP and PLWP were within the limits as described by Deloire and Heyns (2011) for moderate water constraints (Chapter 2). The average SWP was higher than -1MPa prior to *véraison* and higher than -1.2MPa post-*véraison*, which is within the guideline for mild to moderate water constraints (Table C1 in Appendix C). The mean PLWP was higher than -0.4 MPa prior to *véraison* and higher than -0.6 MPa post-*véraison* with the exception of the last two weeks in March 2012, when further irrigation would not have been beneficial so close to harvest. Figure 3.4 depicts more clearly the kinetic of SWP and PLWP,

as well as water inputs (irrigation) during the growing season; the means indicate the aims for mild–moderate water constraints were reached.

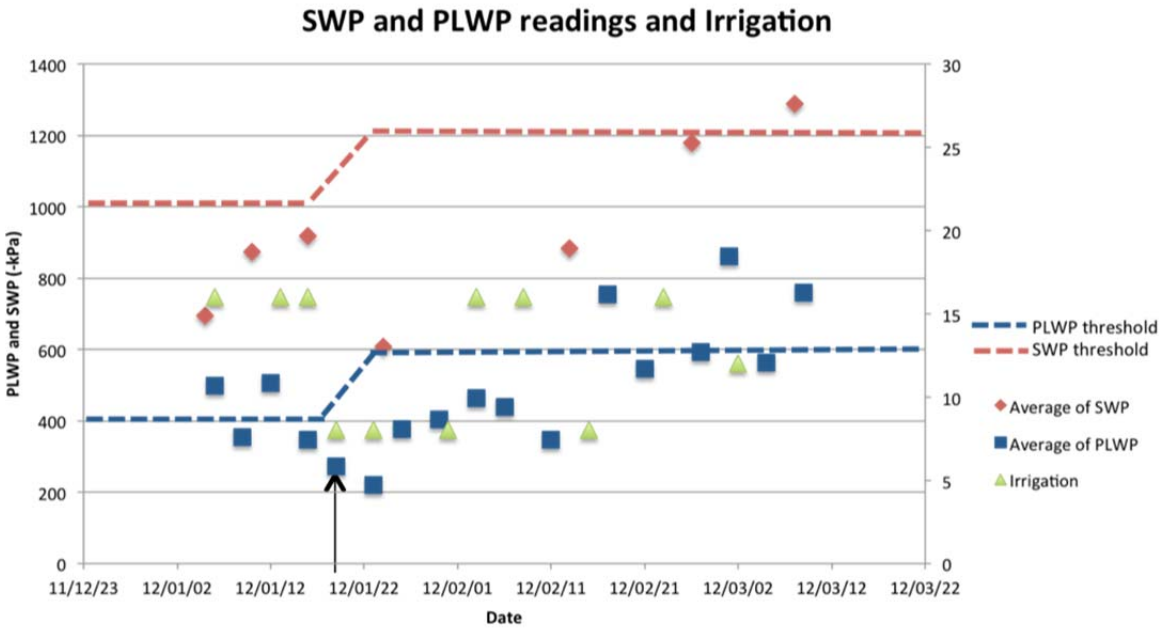

Figure 3.4: SWP and PLWP readings and dates when irrigation was applied during 2012. The arrow indicates *véraison.*

The differences in SWP in January 2012 prompted the division of the block in to low and high vigour zones for sampling and winemaking (Table C2 in Appendix C). Panels 5–8 had a mean SWP of -0.862 Pa and were grouped as Low Vigour (LV) compared to panels 11–14 with a mean of -0.682 Pa grouped as High Vigour (HV). Panels 9 and 10 were used as buffer panels and not used for sampling or winemaking.

The SWP and PLWP deficit increases during the season (Choné *et al.,* 2001). They also showed the relevance of using SWP as an indicator for irrigation scheduling, as the plant response to irrigation is much faster compared to PLWP, which is why readings of PLWP in the Durif vineyard were only taken 2–3 days after irrigation was applied.

The evolution of SWP and PLWP is in agreement with the findings of Choné *et al*. (2001). Choné *et al.'s* (2001) experiment in Bordeaux compare two vineyards— a deep and a shallow soil type. Initially there were significant differences in SWP. As the water deficit became more severe, PLWP also showed significant differences with the shallow soil, a much greater deficit. After heavy rainfall there were no further differences in PLWP, only in SWP continued to show a deficit. Choné *et al.* (2001) explains that the small water deficit is restored by the soil overnight. At midday, when transpiration is at its peak and the transpiration exceeds uptake, the SWP highlights differences in water constraints that would not be identifiable if only comparing PLWP.

This study provides relevant information in understanding the vigour differences and PLWP and SWP readings in the 2012 vintage. The PLWP means remain constant as the irrigation needs are continuously topped up. However, looking at the SWP, initially in the growing season, the low vigour vines have a larger deficit. This could be attributed to differences in soil and water retention capacity early in the season, the lower vigour vines on a steeper slope with faster soil lateral movement of water and greater percentage of runoff. As the season continues the high vigour vines have a larger deficit. This could be attributed to the larger canopies and higher yield of the vines leading to the transpiration rate exceeding the uptake by the roots at midday and restored and therefore not reflected by PLWP as in the case with Choné *et al*. (2001).

In a study on gaseous exchange rates, it was found the low vigour vines have photosynthetic rates 40% lower than high vigour zones before *véraison* (Zerihun *et al*., 2010). It is suggested that low vigour vines have a higher source to sink ratio than high vigour vines and it is proposed that a low demand by the sink limits the rate of photosynthesis. The study explains that water use efficiency can differ with vigour (Zerihun *et al*., 2010).

3.3.2 Homogeneity of the vineyard plot: NDVI and canopy characteristics

The plot used consisted mainly of class 9 and class 10 (yellow and orange) with mean NDVIs of 0.305 and 0.356 respectively (Table 2). Class 9 has a larger area 0.63 (2012) compared to 0.36 (2013) and Class 10 is quite similar 0.24 (2012) and 0.29 (2013). These differences on this site indicate there is more variation between vintages in the lower vigour sections of the block than the high vigour sections of the block. The 2013 results are discussed in detail in Chapter 5. The lower NDVI values on road boundaries could be due to dust from the roads. The NDVI highlights the difference in vigour in the selected plot and confirms the need for repartitioning into two vigour zones (Figure 3.5).

Table 2: NDVI results 2012

Class	Pixels	Area	NDVI				CV,%
			Minimum	Maximum	Mean	SD	
Total for whole block	9 945	0.99	0.175	0.472	0.358	0.029	8.00
8	1 213	0.12	0.175	0.334	0.305	0.027	9.00
9	6 335	0.63	0.334	0.373	0.356	0.010	2.82
10	2 397	0.24	0.373	0.472	0.390	0.017	4.32

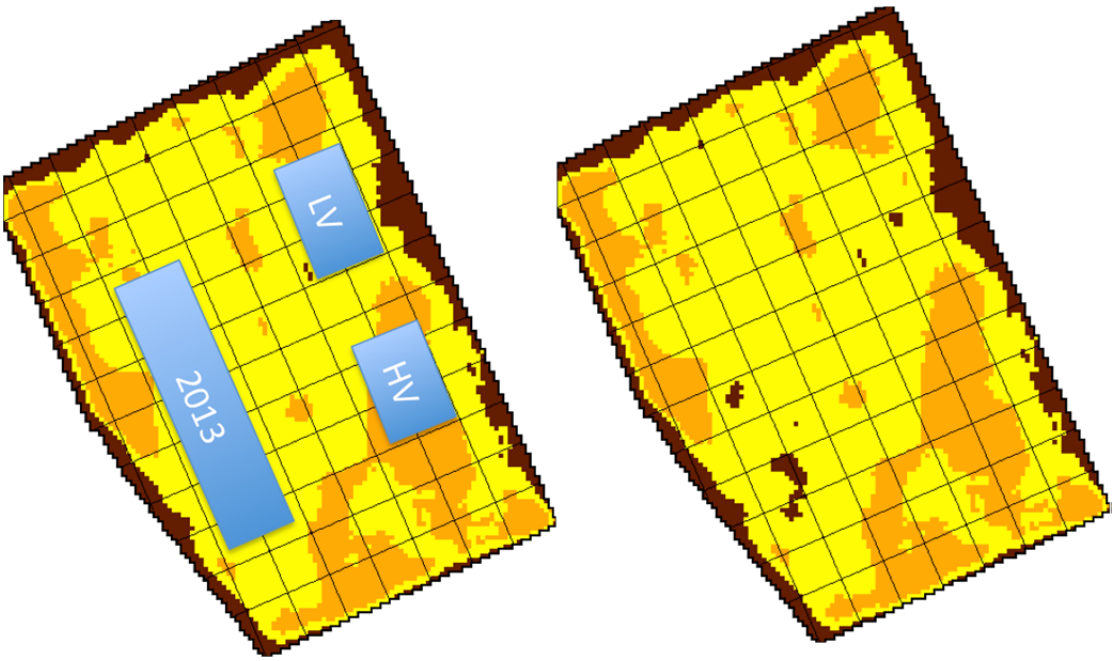

Figure 3.5: Hyperspectral image and plot layout

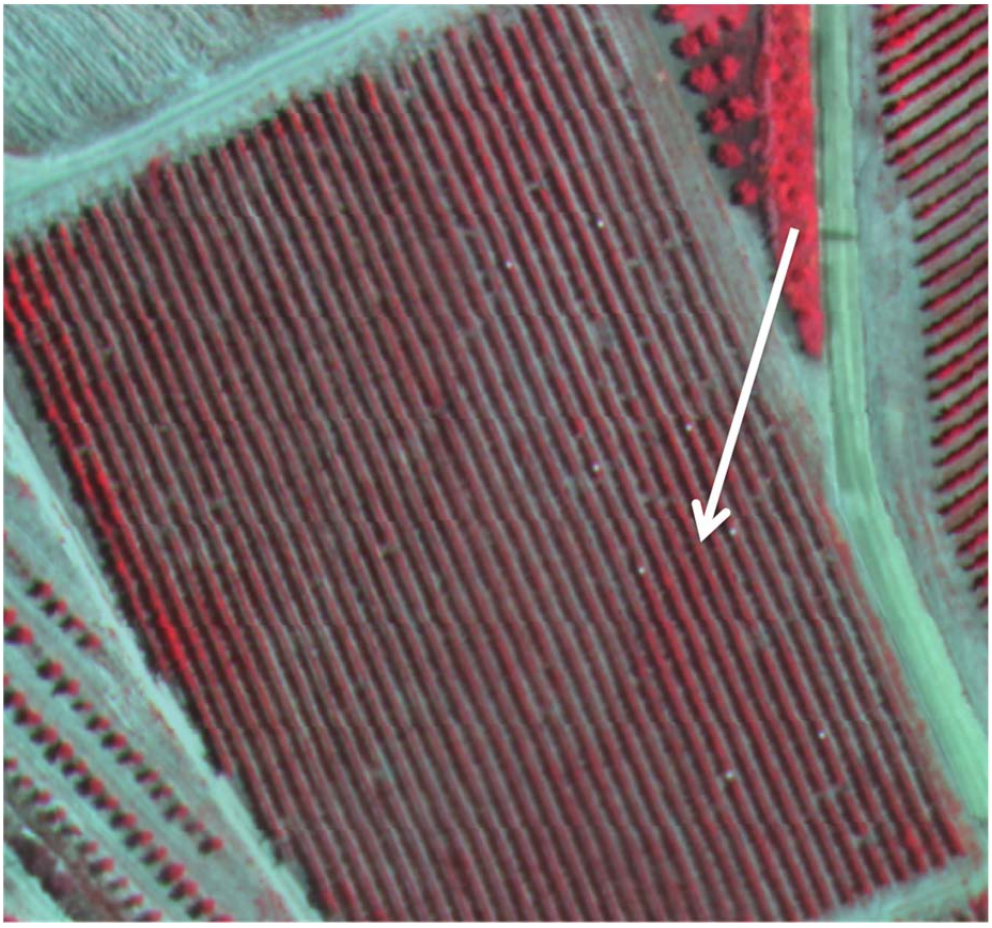

Figure 3.6: NIR with arrow indicating water flow from garden

The NIR shows the apparent runoff of water from a triangular garden at the top of the block (Figure 3.6). The water flow is in the direction of the high vigour area.

3.3.3 Wines and sensory analysis

Due to the nature of the layout of the 2012 plot, the wines and sensory results will be discussed first followed by the relevant analysis. Wines were made from each plot and two to four wines from different harvest dates were made for each treatment. The wines were put through sensory analysis by a panel and the frequency of citation method was used.

Wine aroma

The panel performance for the aromatic descriptors was calculated using the mean R_i which was equal to 0.33 ± 0.10 which is acceptable according to the literature (Campo *et al.,* 2010). Based on *Ri* indices, the number of panelists was reduced from 34 to 31.

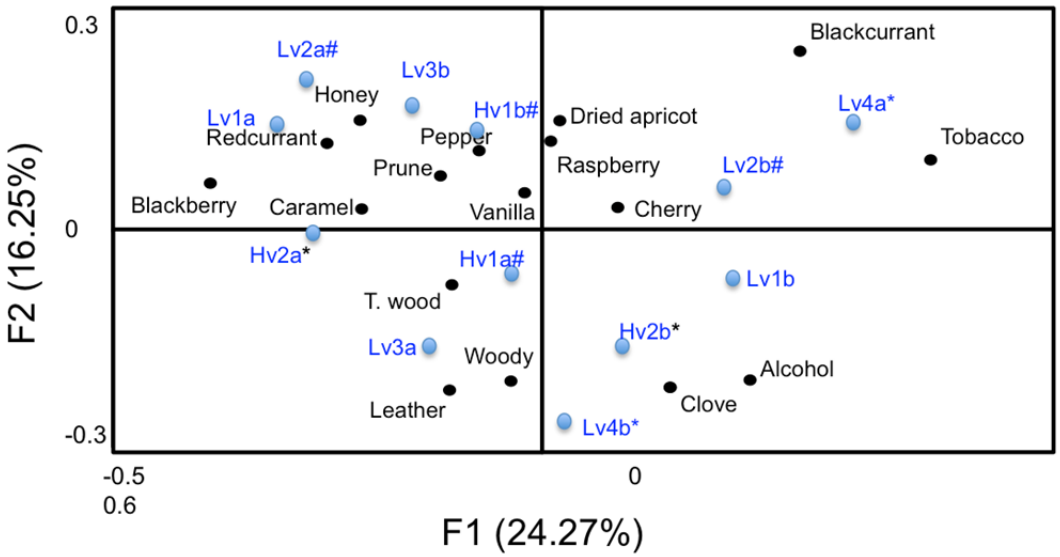

Figure 3.7: Correspondence analysis explaining 40.52% of the variation

The correspondence analysis could explain 40.52% of the variation (Figure 3.7). As there were no biological repeats and several harvest dates, there were no obvious explanations for clusters. The hierarchical cluster analysis divided the wines into the following groups:

- Group 1 (Hv1a#, Hv2a*, Hv2b*, Lv4b*, Lv3a) described by woody/planky, leather, alcohol and clove attributes.
- Group 2(Lv1b, Lv2b#, Lv4a*) described by alcohol blackcurrant, tobacco and clove.
- Group 3 (Hv1b#, Lv1a, Lv3b, Lv2a#,) described by caramel, blackberry, dried fruit.

Three points should be taken into account when interpreting the data; the panel was not globally repeatable, the 2012 wines were one year older when tasted compared to 2013 wines and there were no winemaking replicates.

Group 1 is described by the attributes which could be considered as part of the "neutral" phase of the BAS. Group 2 contains attributes from both neutral (spice, tobacco) and fruit stages (blackcurrant). A wine from Group 2, Lv2b#, also showed a good association with cherry, dried apricot and raspberry attributes. Group 3 could potentially be part of the FF or MF stages, as these wines showed a good association with black berry, prune, redcurrant, caramel, pepper and dried fruit. One could hypothesise, bearing in mind the limitations of the results, Hv1b#, Lv1a, Lv3b, Lv2a# (group 3) and Lv2b# represent the 'fruity' window period for Durif.

The results of the CA show that there is a difference in wine profile between high vigour and low vigour vineyards and that the harvest dates and vineyard treatments have an effect on wine profile. For example, wines from the same harvest date and treatment but with different vigour (Lv2a#, Hv1a#) have different attributes. Two wines from the same treatment and same vigour picked 10 days apart (Lv1a, Lv2a#) showed a strong association with similar attributes – redcurrant, honey, caramel and dried fruit. The next harvest, 3 days later (Lv3a), was associated to woody, toasted wood and leather attributes. The next harvest, 7 days after harvest 3 (Lv4a*), showed an association with blackcurrant and tobacco.

Table 3: Wine codes used for correspondence analysis

Wine code	Harvest number	Harvest date	Treatment
Hv1a#	1	27/02/2012	FV HV
Hv1b#	1	27/02/2012	HV
Hv2a*	2	09/03/2012	FV HV
Hv2b*	2	09/03/2012	HV
Lv1a	1	17/02/2012	FV LV
Lv1b	1	21/02/2012	LV
Lv2a#	2	27/02/2012	FV LV
Lv2b#	2	27/02/2012	LV
Lv3a	3	2/03/2012	FV LV
Lv3b	3	05/03/2021	LV
Lv4a*	4	09/03/2012	FV LV
Lv4b*	4	09/03/2012	LV

Wine mouthfeel

Table 4: Three-way ANOVA for sweetness, sourness, bitterness, astringency and alcohol, where p-value is significant p<0.05

	DF	Sweetness	Sourness	Bitterness	Astringency	Alcohol
Wine	11	0.564	2.850	3.479	12.781	6.747
		0.858	0.001	<0.001	<0.0001	<0.0001
Repeat	1	3.695	0.005	0.032	2.461	0.630
		0.055	0.946	0.857	0.117	0.428
Judge*Wine	396	1.327	1.0	0.843	1.074	1.012
		0.002	0.499	0.955	0.240	0.452

The three-way ANOVA in Table 4 shows that the judges could discriminate between wines for sourness, bitterness, astringency and alcohol, as the p-value is <0.05. Significant sweetness was not significant for the wine factor and was significantly different for the Judge*wine factor indicating the judges were in disagreement regarding sweetness. Sweetness has therefore been taken out of further statistical analysis. The RS range for the wines was 1.07 g/l to 1.66g/l so the differences in perception could also be quite small, complicated by the effect of astringency on sweetness perception. Judges were repeatable in their scoring for all five factors as there were no significant repetition effects.

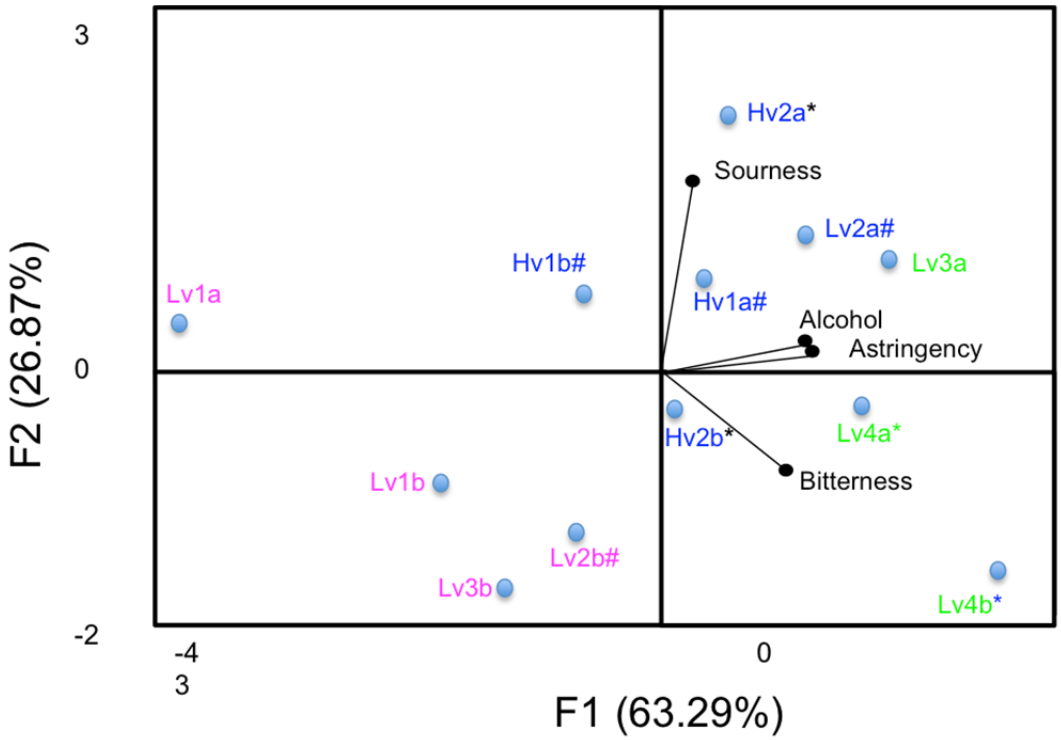

Figure 3.8: PCA bi-plot of wines and attributes. Wines with the same colour indicate the same group in hierarchical cluster analysis

The wines that showed the highest intensity for alcohol and astringency were grouped (Lv4a* and Lv4b* and Lv3a) where Lv3a had a greater sourness intensity rating. Lv4a* and Lv4b* shared the same harvest date — the latest harvest for the Low vigour vines (H4) — and corresponded to the two highest tannin analysis and alcohol in the respective wines (703.47 mg/L, 15.3% and 663.58 mg/L, 15.16%). In terms of aroma attributes, Lv4a* and Lv4b* wines were highly cited for woody/planky and leather attributes and Lv3a was associated with tobacco and blackcurrant. These astringent wines with highly cited woody aromas are in agreement with Cliff *et al*. (2012) who found increasing astringency with grape seed extract led to a decrease of fruity aroma and an increase in woody and earthy aromas. It is possible that the fruit perception is hidden by other attributes and can be masked by vegetative odours and affected by the wine matrix *(*Bindon *et al.,* 2013*;* Escudero *et al.,* 2007).

The wines with the lowest intensity ratings for astringency, bitterness and alcohol were grouped (Lv1a, Lv1b, Lv2b#, Lv3b). In this group Lv1a was rated the lowest for these attributes and higher for sourness – corresponding to the earliest picking date. The lowest perception of astringency and alcohol of Lv1a corresponds to the lowest tannin and alcohol concentration in wine (69.72 mg/l and 12.36%). In terms of aroma, three of these wines (Lv1a, Lv3b and Lv2b#) were in the same group profile and were strongly associated with fruity aromas. Lv1b, which was not in the same aroma group, was closely associated with alcohol on the nose but not on the palate – with alcohol analysis relatively low at 13.38%. A wine which was in the same aroma group (Hv1b#) but which did not fall into the same grouping for the palate, this wine's association to bitterness, alcohol and astringency was similar to the rest of the group but it is described as more sour (corresponding to the wine TA analysis of 6.33, the third highest TA). The correspondence of the wines (Lv1a, Lv3b and Lv2b#) with higher citations for fruity aromas and lower intensity ratings of alcohol, bitterness and astringency is in agreement with the findings of Cliff *et al.* (2012) and Goldner *et al*. (2011). When wines are compared according to harvest timing (and not vigour or treatment) the first harvests have a significantly lower tannin concentration compared to Harvest 4 (Table 8). Literature has shown that fruity aroma is inversely related to astringency and bitterness (Sáenz-Navajas *et al*., 2010). In this case, the wines with the highest citations of red fruit have the lowest perceived astringency.

The last group represents the wines with the highest intensity rating for sourness: Hv1a#, Hv2a* and Lv2a#. The wines made from the vineyard treatment of leaf removal in general have a higher intensity rating for sourness compared to the lateral shoot removal treatment. The wine TAs were 6.33, 6.37 and 6.19, which is relatively higher than the lowest TA of 5.25 of wine Lv3a. The pH of this group ranges from 3.62 to 3.74 and falls within the range of all the wines 3.54–4,02.

3.3.4 Sequential harvesting, must and wine composition

The wines were grouped according to harvest timing (Harvests 1 to 4) and vigour, treatment and wine composition was compared (Table 5).

Table 5: Groups by harvest number, i.e. the first harvest for each plot was grouped together, second harvest together etc. This means the harvest dates were not necessarily the same, but trends between early and later harvests can be observed.

Harvest number	Wine code	Harvest date	Vigour (treatment)
1	Hv1a#	27/02/2012	FV HV
	Hv1b#	27/02/2012	FV HV
	Lv1a	17/02/2012	FV LV
	Lv1b	21/02/2012	LV
2	Hv2a*	09/03/2012	FV HV
	Hv2b*	09/03/2012	FV HV
	Lv2a#	27/02/2012	FV LV
	Lv2b#	27/02/2012	LV
3	Lv3a	2/03/2012	FV LV
	Lv3b	05/03/2021	LV
4	Lv4a*	09/03/2012	FV LV
	Lv4b*	09/03/2012	LV

The must Brix and pH was significantly different for each harvest, increasing from H1 to H4. The TA is only significantly higher for H1 (Table 6).

Table 6: Must Brix, pH and TA

Harvest	Brix	pH	TA
	Standard error =0.34	Standard error = 0.01	Standard error = 0.10
1	22.05^{d}	3.5^{d}	5.73^{a}
2	23.7^{c}	3.59^{c}	5.25^{b}
3	24.64^{b}	3.71^{b}	5.06^{b}
4	26.65^{a}	3.88^{a}	5.08^{b}

The wine pH and TA show no significant differences after fermentation despite significant differences in pH before fermentation (Table 7). The alcohol increases with a later harvest date, which corresponds to the higher Brix for later harvests. The wine RS increases from H1 to H4 and H1 and H4 are significantly different, this corresponds to findings in sequentially harvested Cabernet Sauvignon (Bindon *et al.,* 2013).

Table 7: Wine pH, TA, alcohol and residual sugar (RS)

Harvest	Wine pH	Wine TA	Alcohol	RS
	Std. error = 0.05	Std. error = 0.18	Std. error = 0.33	Std. error = 0.07
1	3.69 ^{a}	5.98 ^{a}	12.87 ^{b}	1.23 ^{b}
2	3.79 ^{a}	5.82 ^{a}	14.19 ^{ab}	1.33 ^{ab}
3	3.8 ^{a}	5.46 ^{a}	14.61 ^{a}	1.3 ^{ab}
4	3.89 ^{a}	5.74 ^{a}	15.23 ^{a}	1.56 ^{a}

There was a trend of increasing tannin concentration with a later harvest date. However, only H4 was significantly different from the rest. This is in agreement with Bindon *et al.'s* (2013) findings of sequentially harvested wines. Durif is well known for its dark and intense colour, and this was supported by the results (Table 8). The total anthocyanins was measured at absorbance at 520 nm (HCl) and ranged from 25.62 to 37 AU. This is far outside the range of Somers and Ziemelis (1985), of 1.3 to 16.2 AU (based on analysis of over 400 wines). According to Ribéreau-Gayon (2000) the range for wine colour intensity is 0.8 to 1.3 AU, in comparison the colour intensity for Durif is very high – 1.2 to 1.8 AU. As expected wine colour density increased in later harvest dates (Ribéreau-Gayon *et al.* 2000) although there was no significant increase after H2. In comparison to Bindon *et al.'s* (2013) results, the trend of colour intensity is similar to an increase in wine colour density with a later harvest date and the values are higher (although this study is on Cabernet Sauvignon, not Durif). There is an increase in hue with a later harvest date, however it is not significant. According to Ribéreau-Gayon (2000), the range for wine colour hue is 0.5 to 0.7 for young wines and can increase up to 1.3 throughout aging. The results range from 0.59 to 0.63 and fall into the range. Degree of red pigment colour showed no significant differences between harvests.

In summary, there was an increase in tannin, total red pigment colour, and wine colour density with a later harvest date. There are significant differences between H1 and H4. The differences between H2 and H3 are smaller and less significant and more obvious trends may have been evident had the wines been made in replicate and the treatments and vigour kept separate.

Table 8: Anthocyanin and tannin analysis of wines

Harvest	Tannin (mg/L)	Total red pigment colour (AU)	Wine colour density (AU)	Wine colour hue	Degree of red pigment colouration (%)
	Std. error = 99.05	Std. error = 1.39	Std. error = 0.57	Std. error = 0.01	Std. error = 1.46
1	215.13 [b]	25.62 [b]	12.01 [b]	0.59 [a]	25.54 [a]
2	430.11 [ab]	30.63 [b]	16.81 [a]	0.6 [a]	29.25 [a]
3	502.54 [ab]	30.74 [b]	15.85 [a]	0.61 [a]	27.44 [a]
4	683.53 [a]	37.51 [a]	18.15 [a]	0.63 [a]	25.23 [a]

3.3.5 Berry ripening

The ripening of the berries of the experimental plot (LV and HV) will be discussed. Sampling of the commercial plot (FV LV and FV HV) did not allow for biological repeats of frozen berries (too few berries were frozen) and therefore the results of glucose, fructose, malic and tartaric acid will not be discussed for this part of the vineyard. Due to the nature of the plot layout changing through the season, the berry analysis was limited to the amount of frozen berries available for processing for each date in order to have technical repeats for statistical analysis. Therefore, the data obtained from frozen berries – glucose, fructose, malic acid and tartaric acid – is presented as means of the LV and HV plots combined. These results cannot be compared directly to SL data or to the wines, however, trends can be observed. Fresh mass and SL data was divided between LV

and HV, and individual curves were used to determine keypoint. The changing of the plot layout during the season added to the complexity of the project and as a result, the 2012 vintage was used to understand the plot better.

5.3.6 Sugar accumulation

The glucose and fructose accumulation (Table 9) are a mean of the LV and HV combined. Fructose and glucose content (mg/berry) followed a similar trend, with no significant difference in accumulation from 3 February 2012 to 17 February 2012; from 21 February 2012 to 9 March 2012 the results are significantly higher than the initial analysis on 3 February 2012. The concentration of glucose and fructose (mg/g berry) increased significantly from 3 to 6 February 2012 and again after 17 February 2012 to 1 March 2012. The increase in concentration was a result of the increasing sugar concentration, as well as the fluctuating fresh mass of the high vigour and low vigour sections. The second increase in sugar accumulation could be due to irrigation (8 mm water) on 15 February 2012 in anticipation of a heat wave, resulting in the reloading of sugar. The reloading was also reflected in the increased glucose to fructose ratio after 17 February 2012.

Table 9: Accumulations of fructose and glucose

	D Fructose (mg per gram berry) Std Error= 2.18	D Fructose (mg per berry) Std Error= 13.62	D Glucose (mg per gram berry) Std Error= 2.50	D Glucose (mg per berry) Std Error= 13.62	Glucose: Fructose
2014/02/03	72.22^{e}	92.59^{c}	73.54^{d}	94.65^{c}	1.02
2014/02/06	85.59^{d}	121.27^{cb}	85.07^{c}	120.71^{cb}	0.99
2014/02/15	92.98^{cd}	130.00^{ac}	91.26^{c}	127.86^{ac}	0.98
2014/02/17	96.20^{c}	120.94^{cb}	90.23^{c}	113.65^{cb}	0.94
2014/02/21	105.93^{b}	146.61^{ab}	102.55^{b}	141.92^{ab}	0.97
2014/03/01	116.23^{a}	168.69^{a}	112.68^{a}	163.74^{a}	0.97
2014/03/09	118.65^{a}	166.93^{ab}	118.85^{a}	165.79^{a}	1.00

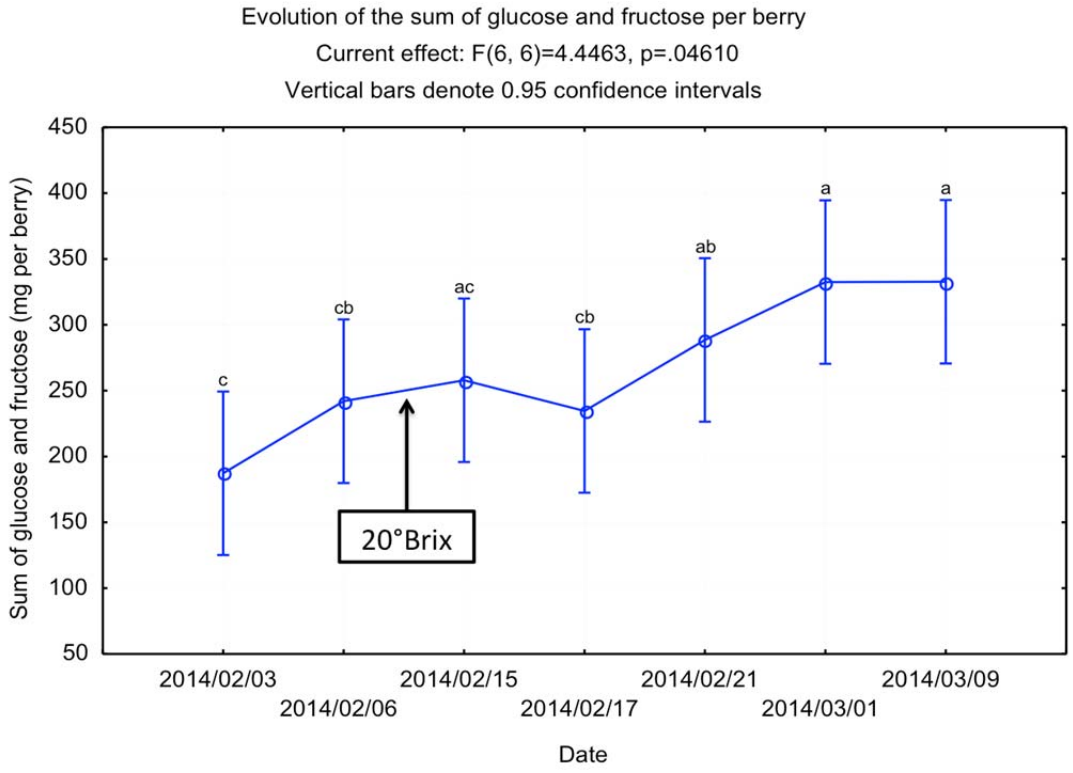

Figure 3.9: The sum of fructose and glucose per berry (HV and LV combined) indicating where 20°Brix average was reached and a mean berry mass of 1.49 g

The LV and HV glucose and fructose content have been added together, in order to be statistically correct, however, this means the effect of the vigour on sugar accumulation is not taken into account. This curve of sugar accumulation is different for each vigour section (LV and HV) and has been displayed in Figure 3.11 and 5.11. In Figure 3.9 the LV and HV have been combined (as though the block had not been partitioned into HV and LV). This could lead to an incorrect estimation of the keypoint and harvest timing. According to Figure 3.9, the keypoint may have been as early as 3 February 2012, as there was no significant difference from 3 February to 17 February 2012, after which the vines started to reload (HV started to reload rapidly at 16mg/berry/day). When compared to Figure 3.12, the difference in sugar accumulation (and therefore vine functioning) between high and low vigour vines is highlighted. This illustrates the importance of having a homogenous sampling area that is representative of the block when using sugar loading as a physiological indicator – or any other method for determining ripeness.

Low vigour compared to high vigour

The difference between sugar accumulation for the high vigour vines (Figure 3.11) and low vigour vines (Figure 3.10) is illustrated. The low vigour vines (Figure 3.10) reached a plateau on 6 February 2012 according to the glucose and fructose analysis, at 210 mg sugar per berry. From 6 to 9 February 2012 sugar accumulation reduced to 4 mg per berry per day. The berry volume on 6 February 2012 represents 78% of the total berry volume. The literature suggests low vigour vines have a higher source to sink ratio than high vigour vines and it is proposed that a low demand by the sink limits the rate of photosynthesis (Zerihun *et al*., 2010). The difference in photosynthetic rates would explain the relatively higher sugar concentration in HV section.

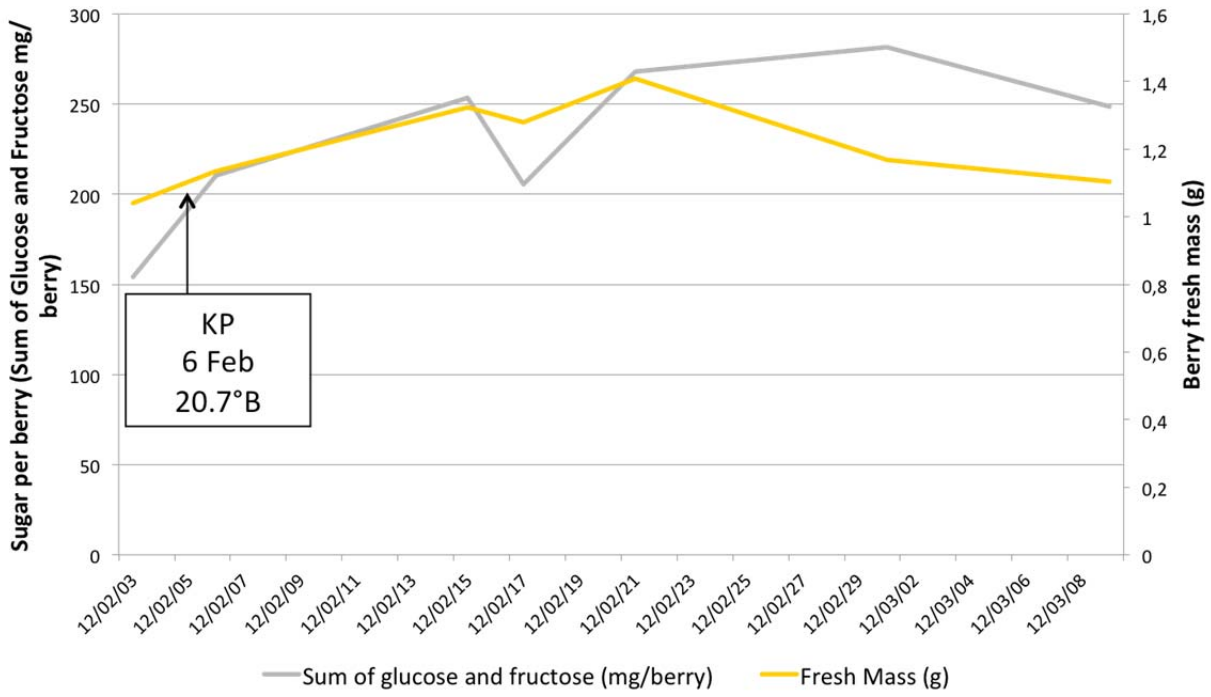

Figure 3.10: Low vigour sugar per berry (sum of glucose and fructose) and fresh mass evolution

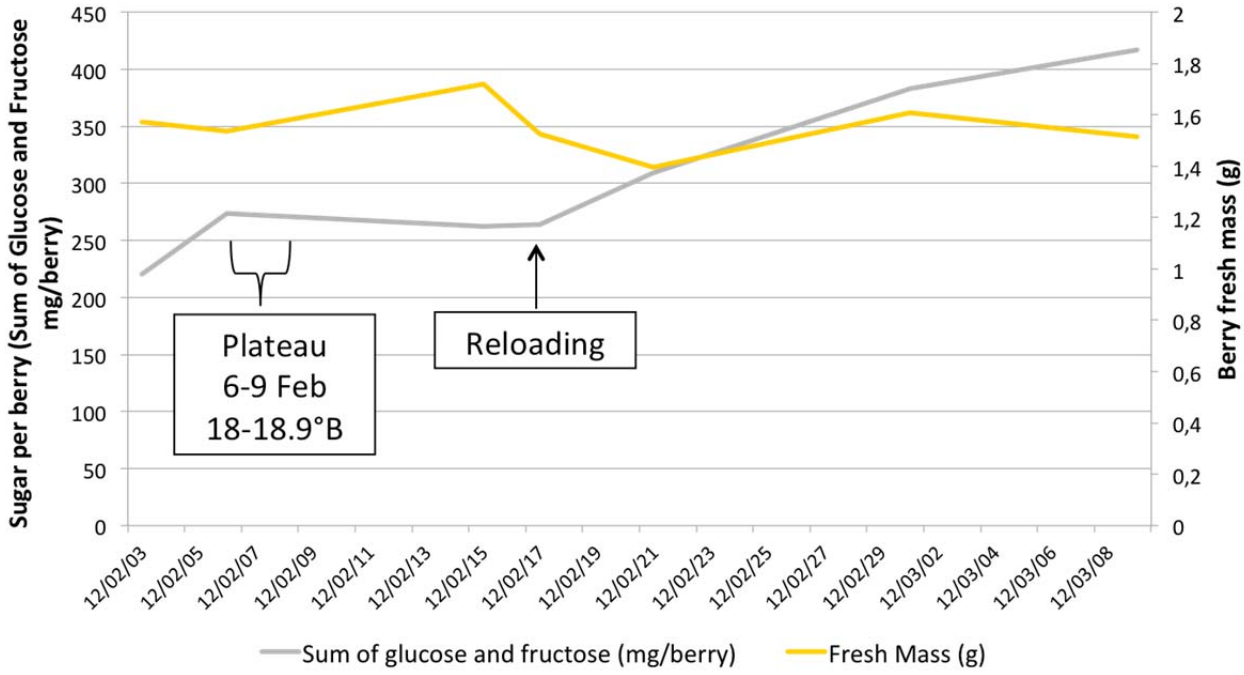

Figure 3.11: High vigour sugar per berry (sum of glucose and fructose) and fresh mass evolution

For the high vigour section of the vineyard, the vines reach a plateau at a sugar level content 30% higher than the low vigour vines (274 mg sugar per berry, 6 February 2012). However, the vines start to reload again after 17 February 2012. The berry fresh mass remains relatively stable and during this period after 17 February 2012 45% of the final sugar content is loaded. There is a good correlation (R^2=0.86) between the sum of glucose and fructose, and the berry fresh mass for the

HV section (Figure C1, Appendix C). The correlation for the LV section of the vineyard is not as good (R^2=0.58). The 2013 data shows a good correlation (R^2=0.78) between the sum of glucose and fructose and the berry fresh mass (Figure B32, Appendix B.2).

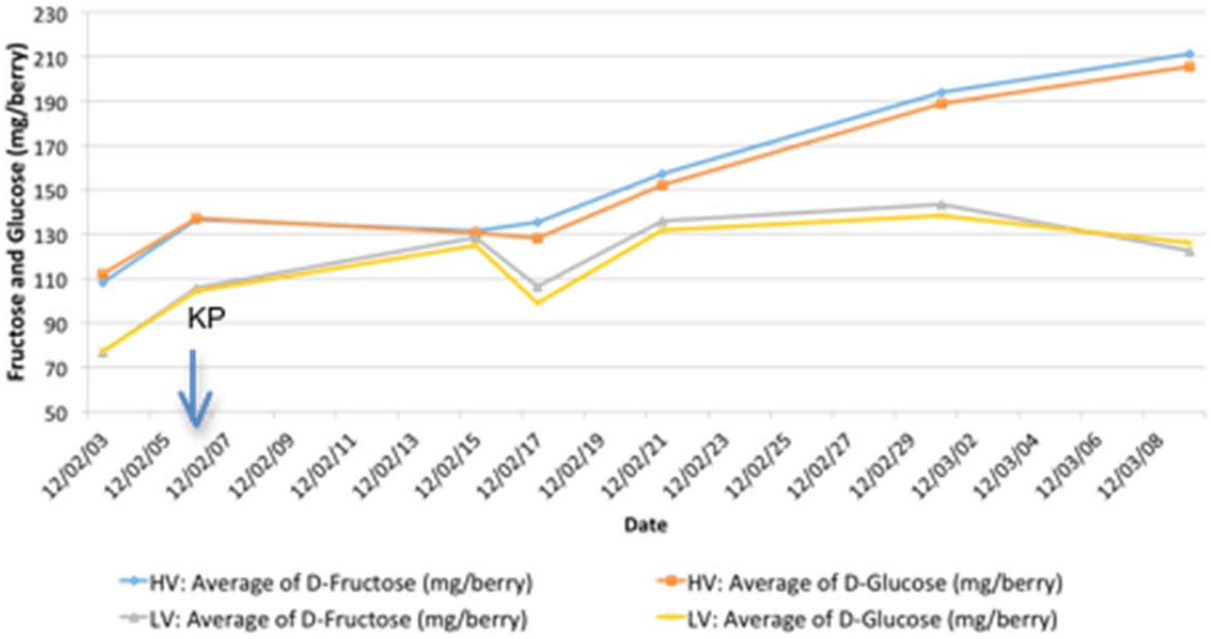

Figure 3.12: Fructose and glucose (mg/berry) accumulation

On comparison to the calculated values, the sugar per berry differs from the actual values. For example, the estimation is 306 mg/berry on 21 February 2012 compared to the actual sum of glucose and fructose, 268 mg/berry. These large differences (23% difference) could be due to the bunch sampling method where bunches are selected and then berries are removed (several berries from one bunch used across bunches) compared to sampling berries in the vineyard across several vines. It would be interesting to compare the potential variability between bunch sampling and berry sampling in the vineyard and the effect the sample size (50, 100 or 200 berry sample sizes) has on the precision on the calculated values.

The second increase in sugar accumulation could be due to irrigation (8 mm water) on 15 February 2012 (1791 degree days), in anticipation of a heat wave, resulting in the reloading of sugar. The effect of this increase in water availability differs between the high vigour and low vigour vineyards (Figure 3.11). The high vigour vines with larger canopies continued to transpire and the fresh mass decreased, sugar concentration increased but the sugar content (mg/berry) remained stable. The low vigour vines' berry fresh mass increased, sugar per berry increased. Although the reloading of sugar seems to be over-estimated when calculated and compared to glucose and fructose analysis, the increase at this point was not significant – suggesting that the sugar accumulation was stable.

Zerihun *et al.* (2010) showed stomatal conductance increased following irrigation and the water use efficiency of LV vines was lower compared to HV vines (Zerihun *et al.,* 2010), and this could explain the reloading of sugar in the HV vines compared to LV. This study also shows that the degree of soil water utilisation differs with vigour. The strong decrease in berry mass for the LV vines could be due to lack of sufficient root density and distribution in low vigour vines (Zerihun *et al.,* 2010). The low vigour vines are on the steeper part of the slope and soil water runoff adds to the problem. The high vigour vineyards, in comparison, receive additional water from drainage from the garden's irrigation and seepage down the slope.

The results of the calculated (estimated) sugar per berry and fresh mass is in Table C3, Appendix C. Again, this emphasizes the need for homogenous plots as high vigour and low vigour vines respond differently to irrigation.

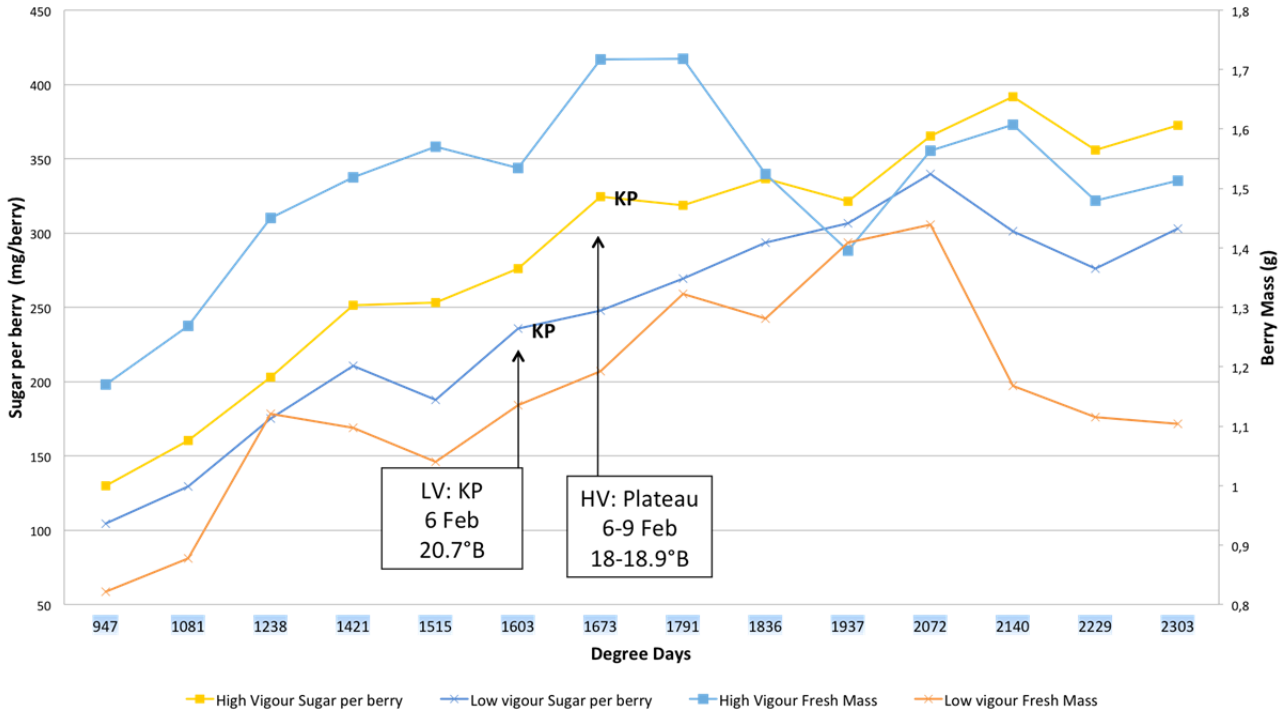

Figure 3.13: Estimated sugar per berry and fresh mass

Sensory analysis and evolution of berry flavours

The low vigour vines reached a plateau on 6 February 2012 at 20.7°Brix, 235 mg/berry (calculated) and 242 mg/berry (sum of glucose and fructose). The high vigour vines reached a plateau between 6 and 9 February 2012 at 17.93–18.85°Brix, 276.1–324.63 mg/berry (calculated) and 274 mg/berry (sum of glucose and fructose). The HV vines, however, started rapid reloading after 15 February 2012 at a rate of 16 mg/berry/day. When sugar accumulation is irregular, 20° Brix could be considered as a potential plateau - from years of expertise using the model worldwide (Deloire, 2013).

The berry flavours evolution is easier to assess, defining harvest windows for fresh and mature fruit stages in vines where berry sugar loading reaches a plateau or slows down (Deloire, 2011,

2013; Antalick *et al.,* 2014). This is evident in the wine styles, as the wines closely associated with "fruity" aromas were Hv1b#, Lv1a, Lv3b, Lv2a# (group 3) and Lv2b# (bearing in mind the limitations of the sensory results) (Figure 3.7, Table 10).

Table 10: Summary of sensory attributes for each wine. The wines in bold show the group of wines with fruit characteristics. The ripening stage* refers to the retrospective assessment of wine styles and harvest windows.

Harvest number	Wine code	Harvest date	Vigour	Wine attributes	Ripening stage* Wine style
1	Hv1a#	27/02/2012	FV HV	woody/planky, leather, alcohol and clove	
	Hv1b#	**27/02/2012 KP+18-21 days**	**FV HV**	**caramel, blackberry, dried fruit**	**Mature Fruit**
	Lv1a	**17/02/2012 KP +11 days**	**FV LV**	**caramel, blackberry, dried fruit**	**Fresh Fruit**
	Lv1b	21/02/2012 KP + 15 days	LV	Alcohol, blackcurrant	
2	Hv2a*	09/03/2012	FV HV	woody/planky, leather, alcohol and clove	
	Hv2b*	09/03/2012	FV HV	woody/planky, leather, alcohol and clove	
	Lv2a#	**27/02/2012 KP +21 days**	**FV LV**	**caramel, blackberry, dried fruit**	**Mature fruit**
	Lv2b#	27/02/2012 KP + 21days	LV	Alcohol and fruit (cherry), blackcurrant	
3	Lv3a	2/03/2012 KP + 24 days	FV LV	Woody, clove, tobacco	
	Lv3b	**05/03/2012 KP+27 days**	**LV**	**caramel, blackberry, dried fruit**	**Overripe**
4	Lv4a*	09/03/2012 KP +31 days	FV LV	Woody, clove, tobacco, blackcurrant	
	Lv4b*	09/03/2012 KP + 31 days	LV	woody/planky, leather, alcohol and clove, tobacco	

The low vigour wines can be assessed in terms of the berry flavour evolution over ripening. The number of days after the keypoint was reached is as follows: LV1a (11 days), Lv2a# (21 days), Lv2b# (21 days), Lv3b(27 days) (Table 3). The LV vines can be considered to show a berry flavour

evolution for Durif with a high citation frequency for fruit characteristics at 11, 21 and 27 days after the decrease in rate of sugar loading.

It would be expected that the wines from the later harvest dates (KP + 31days) showed overripe characteristics, particularly in the LV section as these had a larger decrease in fresh mass. However, these wines (Lv4a* and Lv4b*) had high citations of woody and tobacco aromas (Figure 3.7). The lack of fruitiness could be due to the high perceived astringency in these two in agreement with Cliff *et al.* (2012).

Retrospectively it could be hypothesized that the harvest windows for Durif for fresh fruit wine styles was at KP +11 days, mature fruit wines styles was at KP +21 days and overripe wine styles was at KP +27 days (refer to ripening stage wine style, Table 10).

5.3.6 Tartaric and Malic Acid evolution

The malic and tartaric acid results are the mean for the entire experimental plot, LV and HV combined (Table 11). There is a significant decrease in malic acid content (mg/berry), until 17 February 2012. Malic acid concentration (mg/g berry) follows a similar pattern. This rapid decrease and then stabilization of malic acid was expected (Coombe, 1992). There are no significant differences for the time period measured between the values of tartaric acid per berry and no significant differences for the time period for tartaric per gram berry. Small fluctuations in concentration are a result of changing fresh mass, which is more evident when the results are viewed in terms of low vigour and high vigour (Figure 3.14, Figure 3.15).

Table 11: Evolution of malic and tartaric acid

	Malic acid concentration (mg/g berry)	Malic acid content (mg/ berry)	Tartaric acid concentration (mg/g berry)	Tartaric acid content (mg/ berry)
	Standard Error= 0.13	Standard Error= 0.32	Standard Error= 0.41	Standard Error= 0.38
2014/02/03	2.08^{a}	2.78^{a}	6.64^{a}	8.53^{a}
2014/02/06	1.64^{ab}	2.41^{ab}	6.05^{a}	8.51^{a}
2014/02/15	1.53^{b}	2.17^{ab}	6.15^{a}	8.60^{a}
2014/02/17	1.35^{bc}	1.69^{ab}	6.59^{a}	8.25^{a}
2014/02/21	1.04^{c}	1.44^{b}	6.14^{a}	8.45^{a}
2014/03/01	0.91^{c}	1.35^{b}	6.17^{a}	8.87^{a}
2014/03/09	0.97^{c}	1.38^{b}	6.22^{a}	8.35^{a}

Low vigour and high vigour

The LV berries show relatively lower content of tartaric acid and malic acid (per berry) than HV (Figure 3.14). However, the tartaric and malic acid concentration (per gram of berry), is similar, except towards the end of ripening where LV tartaric acid is relatively higher than HV which decreases (Figure 3.15). This could be due to the relatively larger decrease in berry fresh mass of

the LV vines compared to a smaller decrease in berry fresh mass of the HV vines (Figure 3.16), resulting in an apparent increase in tartaric acid due to berry dehydration and concentration.

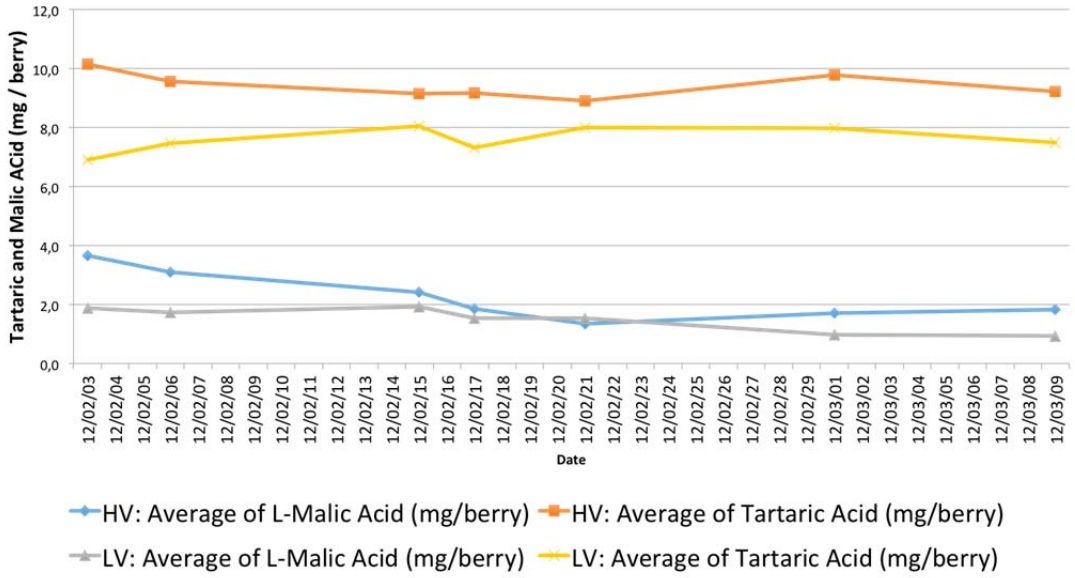

Figure 3.14: Tartaric and malic acid content (mg/berry) for high and low vigour vines

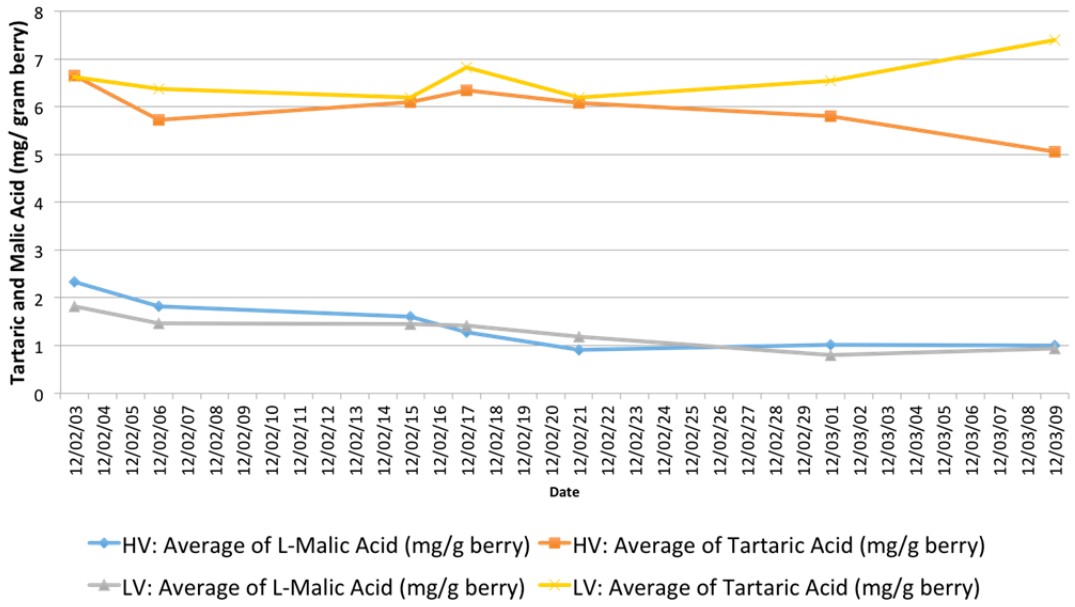

Figure 3.15: Tartaric and Malic acid per gram berry of high and low vigour vines

3.4 Conclusion

The decision to divide the experimental plot into different vigour zones was supported by NDVI images, SWP and PLWP readings, and monitoring sugar accumulation and berry fresh mass. The differences shown between the high vigour vines and low vigour vines highlight the importance of having a homogenous plot for research and for commercial use, and where a homogenous plot is not always possible, to know how the vineyard block is divided, *i.e.*, what percentage is low or high vigour in order to know how to schedule your irrigation and where to sample to determine

optimal harvest times. The results of the NDVI were used to select a more homogenous experimental plot for 2013 in terms of canopy vigour (which does not necessarily mean greater homogeneity in terms of fruit development and composition). Hopefully in the future, with the increasing trend towards precision viticulture, the assessment of vineyard homogeneity, measurement of vine balance, vine water status and fruit quality could become a regular practice (commercially), as this information is much needed for effective decision-making.

The decision to divide the experimental plot into different vigour zones, without allowing enough biological repeats, made the linking of grape composition to wine composition and sensory analysis difficult. This has, however, been shown in the literature to be a complex subject and sequential harvesting complicates this further as the role of ethanol (increasing in wines from later harvests), extractability of anthocyanins and tannins or other compounds like amino acids, lipids, polysaccharides, the latter influencing the chosen yeast metabolism, play an important part in wine composition (Cadot *et al.,* 2012; Bindon *et al.,* 2014; Antalick *et al.,* 2015; Bindon *et al*., 2013). The evolution of the volatile and non-volatile matrix does not always correlate to the ripening evolution of the berry and the associated wines, and does not correlate directly to wine alcohol content (Bindon *et al.,* 2014; Antalick *et al.,* 2015; Bindon *et al*., 2013; Šuklje *et al.,* 2014).

The results of the sensory analysis and SL can be assessed retrospectively. The LV vines reached a plateau on 6 February 2012 (20.7°B) and the HV vines reached a plateau between 6 and 9 February 2012 (18–18.9°B). The HV vines, however, started rapid continuous reloading after 15 February 2012 and therefore did not display a typical plateau curve of berry sugar accumulation. The evolution of berry flavours is only relevant in vines where sugar loading reaches a plateau or slows down (Deloire, 2011). The LV wines can be considered to show an evolution of berry flavours for Durif and had a high citation frequency for fruit characteristics at 11, 21 and 27 days after the KP. Retrospectively it could be hypothesized that the harvest windows for Durif corresponding to fresh fruit wine profiles was at KP +11 days, mature fruit wine profiles was at KP +21 days, and overripe wine profiles was at KP +27 days. This is one of the major finding of this study linked to the calibration of the model and developing a clear understanding of Durif ripening flavours evolution and the corresponding wine styles. This was further developed in the 2013 season.

The mouthfeel of the wines showed an increase in perceived astringency and alcohol perception with an increase in harvest date, which is in agreement with the tannin analysis and Bindon *et al.*'s (2014) findings on Cabernet Sauvignon.

The results of the sensory analysis showed that fruity Durif wines with lower perceived astringency can be harvested earlier at a lower Brix level. These encouraging results inspired the Fairview Winery team to use this approach commercially in the 2013 and 2014 vintages. In 2013 Fairview, together with Vivelys (www.vivelys.com), piloted a project to harvest Durif at fresh fruit and increase the mouthfeel of wines using micro-oxygenation. Trials have been done to monitor the rate of anthocyanin and tannin extraction during fermentation.

Picking date remains an important part of the development of Durif wine styles at Fairview Winery. Despite the challenges, a lot of valuable practical experience and scientific results were gained during the 2012 vintage, which was the first year of the study and the first worldwide calibration of the model using sequential harvest to determine for Durif the harvest sweet spots and the related potential wine styles.

3.5 Bibliography

Antalick, G., Suklje, K., Blackman, J.W., Meeks, C., Deloire, A. and Schmidtke, L.M., 2015. Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry,* 63(18): pp. 4664-72.

Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M. and Francis, I.L., 2014. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry,* **154**(0), pp. 90-101.

Bindon, K., Varela, C., Kennedy, J., Holt, H. and Herderich, M., 2013. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry,* **138**(2–3), pp. 1696-1705.

Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V., 2012. Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. *Analytica Chimica Acta,* **732**, pp. 91-99.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference,* **21**(1), pp. 44-55.

Choné, X., Van Leeuwen, C., Dubourdieu, D. and Gaudillère, J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. *Annals of botany,* **87**(4), pp. 477-483.

Cliff, M.A., Stanich, K., Edwards, J.E. and Saucier, C.T., 2012. Adding grape seed extract to wine affects astringency and other sensory attributes. *Journal of Food Quality,* **35**(4), pp. 263-271.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. *American journal of enology and viticulture.,* **43**(1), pp. 101.

Deloire, A., 2011. The concept of berry sugar loading. *Wynboer,* **257**, pp. 93-95.

Deloire, A., 2013. Berry ripening and wine aroma. *Practical Winery and Vineyard,* pp. 64-74

Deloire, A., 2013. *Wine aroma and grapevine berry ripening: How to capture the complexity. Winetitles, Wine & Viticulture Journal 28(5).*

Deloire, A. and Heyns, D., 2011. The leaf water potentials: Principles, method and thresholds. *Wineland,* 265, pp. 119-121.

Eichhorn, K. and Lorenz, D., 1977. Phenological development stages of the grape vine. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes,* 29(8), pp. 119-120.

Escudero A, Campo E, Fariña L, Cacho J,Ferreira V, 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry,* **55**(11), pp. 4501-10.

Goldner, M., Di Leo Lira, P., Van Baren, C. and Bandoni, A., 2011. Influence of polyphenol levels on the perception of aroma in Vitis vinifera cv. Malbec wine. *S.Afr.J.Enol.Vitic,* **32**(1),.

Hagerman, A.E. and Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry,* 26(4), pp. 809-812.

Lloyd, D., 1990. A phenological classification of terrestrial vegetation cover using shortwave vegetation index imagery. *Remote sensing,* **11**(12), pp. 2269-2279.

Nell, M., 2015. *Sensory characterisation of several red cultivar (Vitis vinifera L.) wines, using berry sugar accumulation as a physiological indicator and sequential harvest,* .

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D., 2000. Handbook of enology. The chemistry of wine and stabilisation and treatments, vol. 2.

South African Wine Laboratories Association (SAWLA), 2002. Methods for analysis for wine laboratories. *South African Society for Enology and Viticulture*.

Sáenz-Navajas, M., Campo, E., FERNÁNDEZ-Zurbano, P., Valentin, D. and Ferreira, V., 2010. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry,* **121**(4), pp. 1139-1149.

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A. and Hammel, H.T., 1965. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. *Science (New York, N.Y.),* 148 (3668), pp. 339-346.

Somers, T.C. and Ziemelis, G., 1985. Spectral evaluation of total phenolic components in Vitis vinifera: Grapes and wines. *Journal of the science of food and agriculture,* **36**(12), pp. 1275-1284.

Šuklje, K., Antalick, G., Meeks, C., Blackman, J.W., Deloire, A. and Schmidtke, L.M., 2014. Optimising harvest date through use of an integrated compositional and sensory model: a proposed approach for better understanding of ripening of autochthonous varieties. *2nd International Oneoviti symposium* November 2014.

Tonietto, J. and Carbonneau, A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology,* **124**(1), pp. 81-97.

Zerihun, A., Lanyon, D. and Gibberd, M., 2010. Vine vigour effects on leaf gas exchange and resource utilisation. *Australian Journal of Grape and Wine Research,* **16**(1), pp. 237-242.

Chapter 4

Climatic and physiological description of the experimental plot and establishing the Durif plot layout

Chapter 4: Climatic and physiological description of the experimental plot and establishing the Durif plot layout

4.1 Introduction

This chapter focuses on describing the experimental vineyard plot of the second year (2013) of this research project. In the first year (2012) of this research project, the plot was not homogenous in terms of vine vigour and therefore in 2013 a homogeneous plot was chosen. A review by Hall *et al.,* (2002) highlights the contribution of vigour to fruit and wine quality and provides an overview of several studies showing the detrimental effects of excessively high or low yield and vigour have on grape and wine quality. The goal in 2013 was to use a homogenous vineyard site (in terms of vine vigour) for sequential harvesting of Durif grapes. The objective was to use hyperspectral imaging, in particular the Normalized Difference Vegetation Index (NDVI), together with other indicators of vineyard homogeneity including, vine water status, pruning mass, leaf area and berry mass, to ensure the site selected was homogenous for winemaking in 2013. The NDVI is used to classify vigour of vegetation (classification in Appendix A). Vigour in remote sensing is used to describe both plant biomass and photosynthetic activity, *i.e.* photosynthetically active plant biomass. It is an important indicator of spatial variation in vineyards and it reflects differences in vegetation that could be due to topography, soil characteristics, water availability, disease, climatic conditions and occurrence of pests (Hall *et al.,* 2002). NDVI is the difference between reflected near infrared (reflected by leaves) and red light (absorbed by chlorophyll):

NDVI = ((near infrared) - (red)) / ((near infrared) + (red))

The index is normalized, therefore it can be used comparatively between sites and times.

Another goal of this research project was to analyse the evolution of Durif fruit composition. The grapes were analysed for sugar (glucose and fructose), acid (tartaric and malic) and anthocyanin content during ripening to show a kinetic of accumulation or degradation of compounds per berry and in concentration.

Vine water status was monitored during the season to ensure the vines were not subjected to more than moderate water constraints (Table 1 and Table 2). Pre-dawn Leaf Water Potential (PLWP) and Stem Water Potential (SWP) measurements using a pressure chamber were used as indicators of vine water status (Deloire and Heyns 2011). The following thresholds (Table 1 and Table 2) have been used in South Africa as ranges to manage irrigation and vine stress but soil structure, canopy size and cultivar should also be considered (Deloire and Heyns, 2011). The classes (Table 2) are recommended based on phenological stage. Budburst - flowering: classes 0 to

1; Pea size - *véraison*: classes 1 to 2; *véraison* - harvest: classes 1 to 4 depending on the yield and style of the wine. Class 5 is to be avoided. The thresholds differ from cultivar to cultivar (Deloire and Heyns, 2011).

Table 1: Simplified Thresholds of PLWP (1 bar = 0.1 MPa = 100 KPa) (Deloire and Heyns, 2011 Wineland issue 265)

Pre-dawn leaf water potential (Ψ_{plwp}, MPa)	*Level of water constraint or stress*
0 to -0.3	Little or no water deficit
-0.3 to -0.6	Moderate to severe water deficit
< -0.6	Water stress

Table 2: Thresholds of SWP for most cultivars and terroir units in South Africa (Deloire and Heyns, 2011 Wineland issue 265)

Classes	**SWP (Ψ_{SWP}, MPa)**	**Level of vine water deficit**
0	≥ -0.6	Zero water deficit
1	-0.7 to –0.9	Mild to moderate water deficit
2	-1.0 to –1.2	Moderate water deficit
3	-1.2 to -1.4	Moderate to important water deficit
4	-1.4 to 1.6	Strong to severe water deficit (possible plant and cell damages)
5	< -1.6	Severe water deficit (stress: plant and cell damage).

The climate is described by three major indices. The Cool Night (CI) or Fresh Night Index (FNI) is the average of the minimum temperature one month before harvest (the chosen period depends on the analytical goals). This gives a qualitative indication of the development of secondary metabolites such as anthocyanins and phenols (Tonietto and Carbonneau, 2004). The four climate classes according to the FNI are indicated in Table 3.

Table 3: Fresh Night Index climate classes (from Tonietto and Carbonneau, 2004)

Warm night	FNI_1 > 18
Temperate night	FNI_2 14 to 18
Cool night	FNI_3 12 to 14
Very cool night	FNI_4 < 12

The heliothermal Huglin Index (HI) is a six month heat summation (the choice of the period depends on the region) of the average daily mean and maximum, minus 10°C, which gives an indication of the climate description during the vegetative cycle (Tonietto and Carbonneau, 2004; Huglin, 1978):

$$HI = ((\text{Daily mean T} - 10\ °C) + (\text{Daily max T} - 10\ °C)) / 2$$

Table 4: Huglin Index classification (Huglin, 1978)

Very Cool	Below 1500
Cool	1500–1800
Temperate	1800–2100
Warm Temperate	2100–2400
Warm	2400–2700
Hot	Above 2700

Winkler Index (Growing Degree-Day) is a method used for classifying grape growing regions using heat summation over the growing season from 1 September to 31 March (Winkler *et al.,* 1974):

Heat summation = sum of the daily Mean Temperature - 10

4.2 Materials and methods

4.2.1 Experimental vineyard

The Durif used for 2013 was the same block as the 2012 study (see Chapter 3.2.2). However, a different part of the block was used.

4.2.2 Climate and climatic indices, temperature and light

A weather station, based 150 meters from the plot, was used to measure rainfall, daily maximum and minimum temperature, average hourly wind speed, and total hours with wind speed greater than 5 m/s. In addition, the following were also measured: daily maximum and minimum humidity, total rainfall, total radiation, total reference evapotranspiration (calculated according to the FAO 56 Formula; mm/day), and water consumption value (mm/day, ET0 values multiplied with Crop Factor). The weather station data was used to calculate Winkler Index, FNI and HI indices (Tonietto and Carbonneau, 2004)

In 2013 a data logger (TinyTag^{TM}) measuring light intensity was positioned to measure exposed and shaded bunches every fifteen minutes from *véraison* to harvest. A mesoclimatic data logger (TinyTag^{TM}) in the canopy was positioned on an exposed and shaded bunch measuring bunch temperature and humidity every fifteen minutes from *véraison* to harvest. The collected data was used to calculate the growing degree-days from 1 December 2012 and 1 December 2013 to the last harvest date.

4.2.3 Experimental plot layout

The experimental plot layout and position within the block was based on the findings of 2012, which highlight differences of vigour within the plot. The hyperspectral image from the 2012 vintage was used as a guideline. The plot was surrounded by a minimum of 4 buffer rows and 4 buffer panels separating the rest of the block from the road. Vines that did not conform or were not homogenous or underperforming were marked and excluded from the study. The plot area

was nine rows by nine panels (five vines per panel between two poles) and this area was further divided into nine blocks consisting of three rows and three panels each. These nine blocks were randomly allocated to make three biological repeat groups which were marked with coloured ribbons in the vineyard and hereafter referred to as the Red (R), Blue (B) and Green (G) biological repeats (Figure 4.1). The colours have no specific meaning and could be considered as biological repeats one, two and three. All sampling and measurements were done per biological repeat (each consisting of 3 blocks), essentially the vineyard information was gathered in triplicate from various places across the vineyard.

Row 1	RED	RED	RED	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 2	RED	RED	RED	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 3	RED	RED	RED	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 4	BLUE	BLUE	BLUE	RED	RED	RED	GREEN	GREEN	GREEN
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 5	BLUE	BLUE	BLUE	RED	RED	RED	GREEN	GREEN	GREEN
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 6	BLUE	BLUE	BLUE	RED	RED	RED	GREEN	GREEN	GREEN
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 7	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE	RED	RED	RED
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 8	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE	RED	RED	RED
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines
Row 9	GREEN	GREEN	GREEN	BLUE	BLUE	BLUE	RED	RED	RED
	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines	5 Vines

Figure 4.1: Plot layout showing biological repeats red, blue, green

4.2.4 Vineyard management

The cordon was pruned to two bud spurs in August 2012. The vineyard was suckered on September 2012 and the shoots were positioned vertically and wires lifted in November 2012. After set (Eichhorn Lorenz stage 29) strong leaf removal (20%) was done on the morning sun side of the canopy between the cordon and the bunch zone of the entire block. *Véraison* was 100% complete the week of 7 January 2013.

Figure 4.2: Grape bunch exposure to light before harvest

4.2.5 Monitoring grapevine water status

Grapevine water status was monitored using a pressure chamber according to the technique described by (Scholander *et al.,* 1965). Pre-dawn Leaf Water Potential (PLWP, ψ_{plwp}) and Stem Water Potential (SWP) were measured in the 2012 and 2013 seasons. In 2012 the irrigation programme at Fairview farm was calibrated using SWP and PLWP, evapotranspiration values and soil water probes. Therefore, in 2013 SWP and PLWP was not required for irrigation scheduling but rather to confirm the water status of the vines at various time points (pre- and post-*véraison*: Eichhorn Lorenz stages 33–38) to ensure the water constraints were only moderate.

In 2013 the SWP was measured three times during the growing season. PLWP was done on 11 January 2013 (post-*véraison*) and again after irrigation on 17 January 2013, thereafter weekly irrigation followed. A final reading was done on 13 February 2013. PLWP was measured for 18 panels and three vines per panel were tested (total of 54 readings per date). The objective was to ensure PLWP was greater than -400 KPa and SWP was greater than -1 000 KPa (mild to moderate water deficit) pre-*vérasion*. Post-*véraison* and closer to harvest, irrigation was used to maintain PLWP greater than -600 KPa and ensure SWP was greater than -1 200 KPa.

4.2.6 Canopy characteristics

Outline Imagery™ took hyperspectral images on 21 January 2013. Data was projected on Universal Transverse Mercator using WGS 84 datum as described in Chapter 3.

In 2012 only NDVI images were used and berry mass was monitored during the growing season. In 2013 NDVI images were used and the following canopy characteristics were measured: bunch mass, berry mass, cane mass, leaf area and shoot length.

Berry mass was determined during the growing season from January to March 2013. Bunch mass was determined on 3 dates whilst harvesting: 1, 3 and 11 March 2013. A minimum of 30 vines was used per biological repeat to determine the number of bunches per vine, mass of grapes per vine and the average bunch weight.

Cane mass was determined in August 2013 at pruning. The canes of a minimum of 21 vines were used per biological repeat to determine the number of canes per vine and cane mass per vine. The cane mass to yield ratio was determined. The cane mass was measured on the same vines the bunch mass was measured.

Shoots were sampled post-harvest to determine leaf area per shoot. Nine shoots were sampled per biological repeat and this was done across three rows and three panels (i.e., one shoot was sampled per panel). The main shoot and lateral shoot length was measured (cm) and the number of lateral shoots, internodes, leaves (lateral and main shoot) and leaf area (main and lateral) was determined using an electronic leaf surface meter (Delta-T Devices, Cambridge, UK). Where shoots were split, this was recorded separately. The lateral shoot length was measured by combining the length of all the lateral shoots on the main shoot.

4.2.7 Monitoring the tempo of ripening

From *véraison* the berry mass evolution and Brix were monitored together with other indicators (pH and TA). Grapes were frozen (-80°C) in order to study the evolution of tartaric and malic acid, fructose, glucose and anthocyanins.

Berry sampling

Berry sampling was done randomly in the vineyard before 10:00 in the morning. Berries were cut at the pedicel. In 2013 only random berry sampling was done in the vineyard and a minimum of 150 berries were sampled per biological repeat for berry fresh mass and 50 berries were frozen (-80°C).

Fresh mass, Brix, pH and Titratable Acidity

Berry mass was done by weighing a known number of berries on a scale, the berries were then crushed and the juice was used to record the Brix using a refractometer (Opti refractometer, United Kingdom). The remaining juice was used to measure pH and titrated with 0.33 N NaOH to determine the Titratable Acidity (TA) (Metrohm 702 SM Titrino, Switzerland). The recording of fresh mass was done immediately after sampling to prevent any desiccation of the berry.

4.2.8 Determining fruit composition

The methods used to determine the evolution of fructose, glucose, malic acid and tartaric acid in berries are described in Chapter 3 (3.2.8).

4.2.9 Statistical analysis

Data was subjected to analysis of variance (ANOVA). Mean comparisons were performed using Fisher's Least Significant Difference (LSD) test ($p \leq 0.05$). Mean comparisons with confidence intervals set at 0.95 were used to show trends (Software: Statistica).

4.3 Results and discussion

4.3.1 Mesoclimatic indices

Winkler Index

Table 5: Winkler Index (GDD) for 2012 and 2013 season

	2011/2012	2012/2013
	GDD	GDD
September	139.755	107.255
October	216.76	205.41
November	237.065	288.605
December	338.82	442.965
January	468.29	417.975
February	364.045	362.515
March	387.8	380.08
Total	**2152.535**	**2204.805**
Winkler Classification	Region IV hot	Region V very hot

According to the Winkler Index, the two vintages in this study fell into two different classifications – hot and very hot respectively.

Huglin results

Table 6: Huglin Heat Summation for 2012 and 2013 growing season

	2011/2012	2012/2013
October	316.42	289.125
November	337.4525	397.7975
December	438.595	545.7875
January	578.585	521.2125
February	465.0825	454.7025
March	493.78	478.205
Total	**2629.915**	**2686.83**
Huglin Classification	Warm	Warm

The warm classification indicates the climate exceeds the heliothermal requirements for ripening (Tonietto, Carbonneau 2004).

Fresh Night Index

2012 results

FNI for January 2012 = 18.45 **Warm night**

FNI for February 2012 = 16.41 **Temperate night**

Table 7: Calculation of FNI every two weeks from *véraison* to harvest

Time Period	FNI	Classification
12/01/01–12/01/14 (pre-*véraison*)	17.1	Temperate
12/01/14–12/01/28 (*véraison* 100% complete)	19.5	Warm
12/01/28–12/02/11	17.8	Temperate
12/02/12–12/02/26	15.5	Temperate
12/02/27–12/03/09 (12/03/09 last harvest)	15.9	Temperate

2013 results

FNI for January 2013 = 16.7 **Temperate night**
FNI for February 2013 = 16.40 **Temperate night**

Table 8: Calculation of FNI every two weeks from *véraison* to harvest

Time Period	FNI	Classification
13/01/01–13/01/14 (pre-*véraision)*	14,35	Temperate
13/01/14–13/01/28 (*véraison* 100% complete)	18,7	Warm
13/01/28–13/02/11	18,24	Warm
13/02/12–13/02/26	15,04	Temperate
13/02/27–13/03/09 (13/03/08 last harvest)	15,83	Temperate

The period of warm night temperatures could affect the aromatic potential and colour development during ripening. The general night temperatures are classified as temperate, which is between warm and cool, this is more favourable for later ripening varieties than early varieties (Tonietto and Carbonneau, 2004).

Table 9: Number of days where daily maximum temperature exceeded 35 °C.

	Number of days	
Month	**2011/2012**	**2012/2013**
November	1	0
December	1	5
January	9	1
February	4	4
March	3	4

The 2012 growing season had more days exceeding 35°C, leading to stomatal closure and decreased photosynthesis.

4.3.2 Light measurements in the canopy

Photosynthetically Active Radiation (PAR) of a shaded bunch inside the canopy and an exposed bunch (where leaves had been removed) was measured. Sunlight and UV exposure to the bunch zone has been shown by Song *et al.*2014) to affect Pinot Noir berry composition (anthocyanins, tannins, Brix and pH) and in turn wine composition (terpene alcohols and C_{13}-norisprenoids, to

name a few). By modifying light quantity (exposed bunches) and light quality (reduced UV radiation) in Sauvignon Blanc, Šuklje *et al.,* (2014) observed changes in thiol concentration in wines and differences in wine styles. In Figure 4.3.3 the PAR is relatively low during the day and increases at 4 pm when sunlight penetrates the canopy due to the NW/SE row direction. Each time period for both Figure 4.3.3 and Figure 4.4 follow similar patterns except for the period 3–12 February 2013 (grey) which has a lower PAR. This could be due to overcast conditions and rain on 9–11 February 2013. Photosynthetic activity is impacted by PAR. The PAR in the exposed bunches (Figure 4.4) is relatively high and could impact the grape composition (carotenoids and flavonoids, to mention a few) (Gregan *et al.,* 2012; Young *et al.,* 2012), IBMP (Scheiner *et al.,* 2010) and volatile composition of wines (Šuklje *et al.,* 2014).

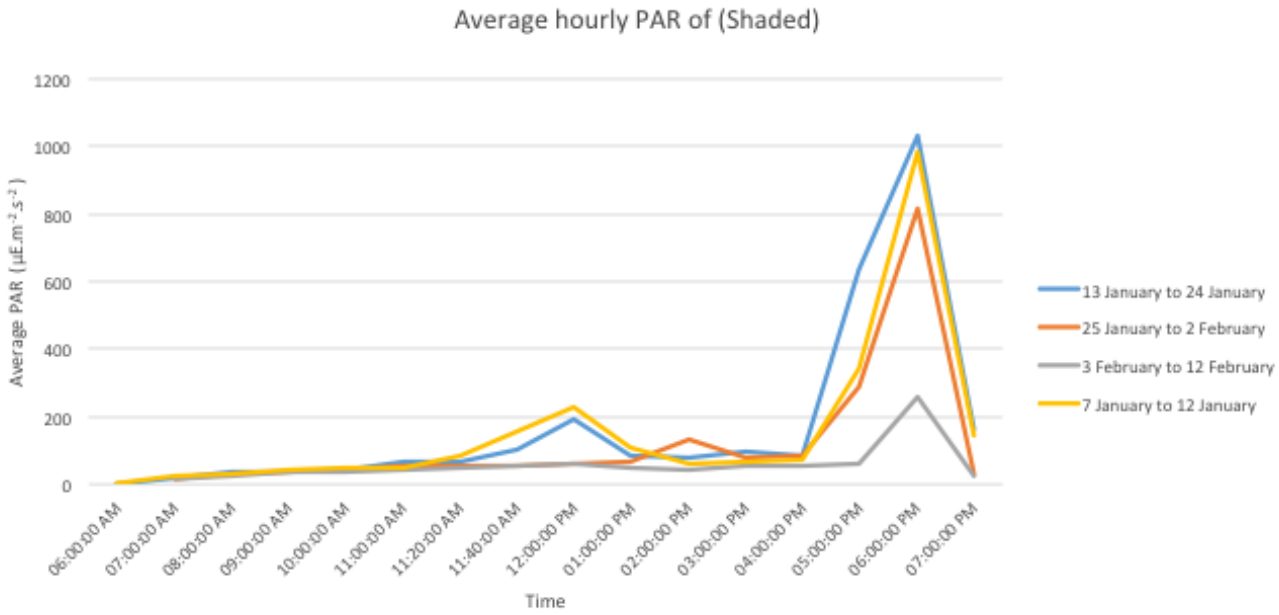

Figure 4.3: Average hourly PAR of shaded bunches over different periods from 13 January to 12 February 2013

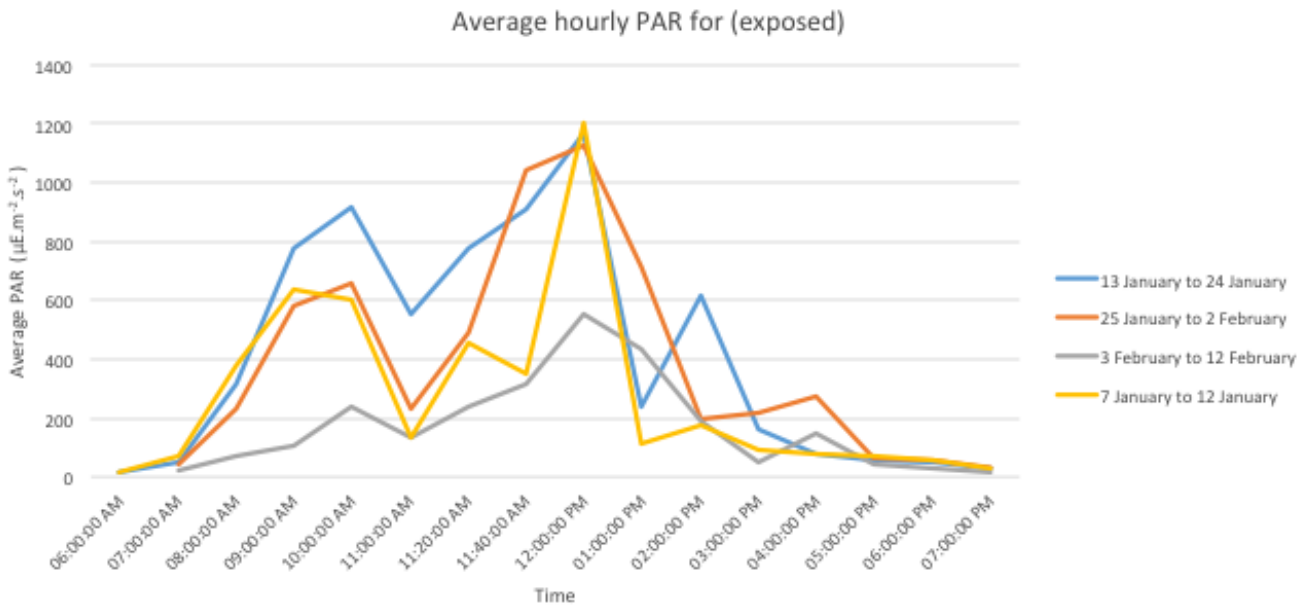

Figure 4.4: Average hourly PAR of exposed bunches over different periods from 13 January to 12 February 2013

4.3.3 Microclimatic measurements

Bunch temperature results

Hourly temperatures of an exposed bunch (morning sun) and shaded bunch (inside the canopy on the afternoon sun side of the canopy) were monitored from 25 January to 9 March 2013 (Eichhorn Lorenz stage 35 to 38). Between 11 and 2 pm the difference in bunch temperature was between 4 °C and 6 °C. The data has been divided into two time periods: early season 25 January to 14 February 2013 and late season 15 February to 9 March 2013. As can be expected, the temperatures were much higher on the exposed bunches in early morning, dropping in the late afternoon faster than the shaded bunches. Average temperatures of exposed bunches were even higher later in the season. Maximum bunch temperatures reached for the time period 25 January to 14 February were 45 °C and 33 °C for exposed and shaded respectively. Maximum temperatures reached over the period 15 February to 9 March 2013 were 44 °C and 36 °C for exposed and shaded respectively.

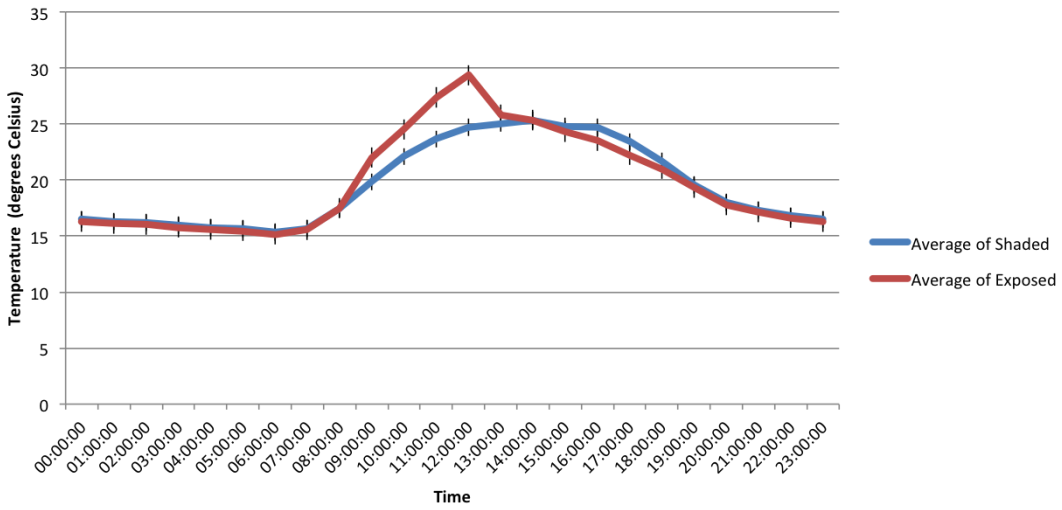

Figure 4.5: Hourly average temperature for exposed and shaded bunches for the period 25 January to 14 February 2013 (Eichhorn Lorenz stage 35 to 36)

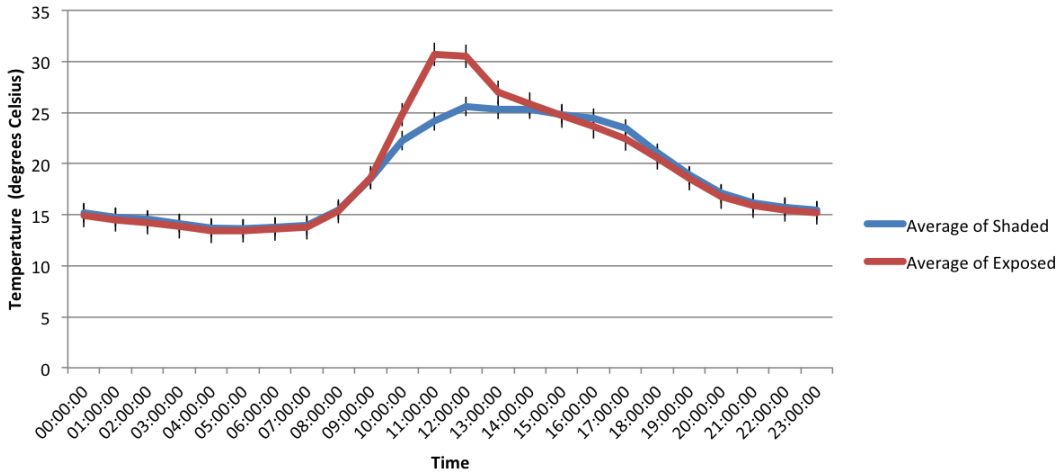

Figure 4.6: Hourly average temperature for exposed and shaded bunches for the period 15 February to 9 March 2013 (Eichhorn Lorenz stage 36 to 38)

4.3.4 Homogeneity of the vineyard plot: NDVI and canopy characteristics

The plot used consisted mainly of class 8 and class 9 (brown and yellow) with mean NDVIs of 0.3 and 0.34 respectively (Figure 4.7, Table 10). There may be small differences across the plot, as can be expected in any natural environment, and therefore — to further reduce the effects of vigour differences — the sample plot was randomly divided into zones (marked with R, G and B) as biological repeats for sampling and winemaking. The variation in the NDVI values on the road boundaries could be due to the effect of dust from the roads impacting on the vine vigour.

Table 10: NDVI Results 2013

Class	Pixels	Area	NDVI				CV, %
			Min	Max	Mean	SD	
Total for whole block	**9 860**	**0.99**	**0.147**	**0.470**	**0.340**	**0.036**	**10.56**
8	3 345	0.33	0.147	0.321	0.303	0.016	5.41
9	3 605	0.36	0.321	0.361	0.340	0.012	3.43
10	2 910	0.29	0.361	0.470	0.384	0.020	5.23

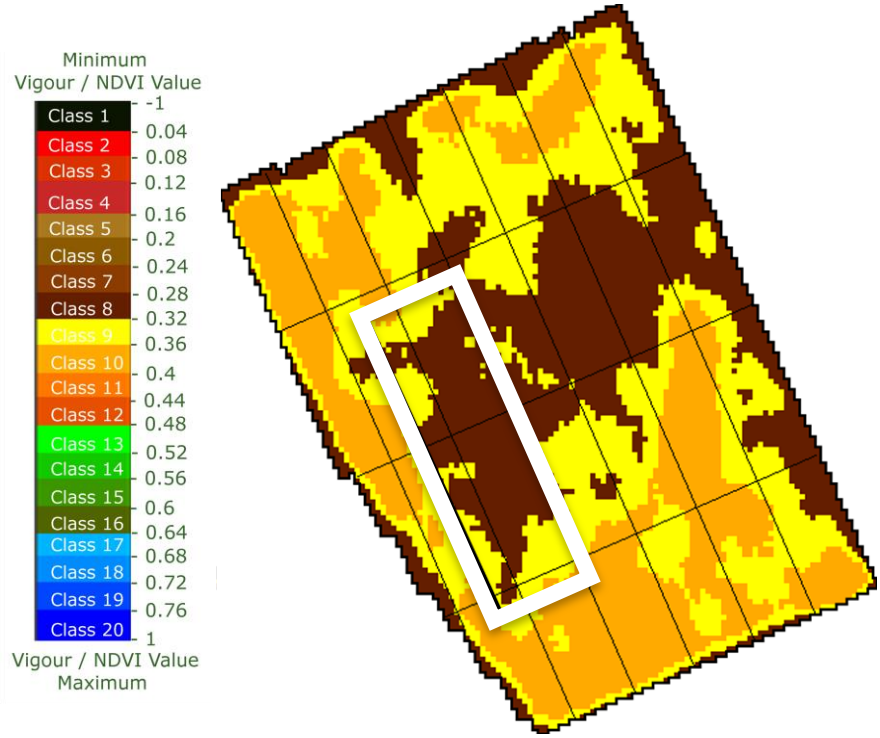

Figure 4.7: NDVI showing vineyard and experimental plot area (2013)

To further emphasize the relative homogeneity or heterogeneity in this experimental plot, and to understand the canopy characteristics, a number of measurements were taken. These included: (1) number of bunches per vine, fresh mass of grapes per vine, mean bunch mass, (2) main and lateral shoot length, (3) number of leaves and leaf area of primary and lateral shoots, (4) number of canes per vine, cane and mass yield to cane mass ratios and (5) leaf area to yield ratio.

(1) The differences in number of bunches per vine, mass of grapes per vine and mean bunch mass were investigated per panel and per biological repeat (Table A1, Appendix A). There were few significant differences across the panels and these were randomly distributed between the biological repeats (Table A2, Appendix A).

(2) There was an increase in main shoot length and a subsequent increase in the number of lateral shoots with increasing vigour (increasing panel number). A relatively small change in

vigour was noticed as well as differences in growth between panels 1 and 9 (Table A3, Appendix A). When combining the data into the groups for biological repeats the only significant difference was for the lateral shoot length (Table A4, Appendix A).

(3) Panels 8 and 9 have the highest leaf area and number of leaves per main shoot (Table A5, Appendix A). This shows there is some variability in vine growth in the last panels (panels 8 and 9), however there was no significant difference in leaf area and number per main shoot between biological repeats (Table A6, Appendix A). The leaf area was measured per shoot and not per vine, so the leaf-area to fruit-mass ratio could not be calculated. An approximation using the number of canes per vine (which was counted during pruning) was used to determine an approximate leaf area per vine (mean per biological repeated). The leaf-area to fruit-mass ratio per vine was equal to 0.6 for each biological repeat, which is below the range of Kliewer and Dokoozlian (2005) (0.8 to 1.2m^2 leaf area per kilogram fruit). This result was however, an approximation and the value of leaf-to-fruit ratio on vine balance and fruit composition as published in numerous articles (Parker *et al.,* 2015, Kliewer and Dokoozlian, 2005, Šuklje *et al.,* 2013) is presently debatable. The main practical goal of canopy manipulation is to alter the fruit zone microclimate (Wessner and Kurtural, 2012; Deloire 2012).

(4) The crop-load to pruning-weight ratio for repeat Red, Blue and Green is 6.17, 6.52 and 6.56, respectively, which is within the optimal range of 5 to 10 (Bravdo et al., 1985). The ratio of cane mass:yield vine (Table A7, Appendix A) shows some significant differences as you move down the row, but when grouped into biological repeats there are no significant differences.

As can be seen from

Figure 4.8, 3.9 and 3.10 the accumulation of fresh mass, sugar and Brix per berry is very similar across all three biological repeats. The behaviour of the vines across the vineyard plot can be considered homogenous. The sudden increase in fresh mass was due to late season rain.

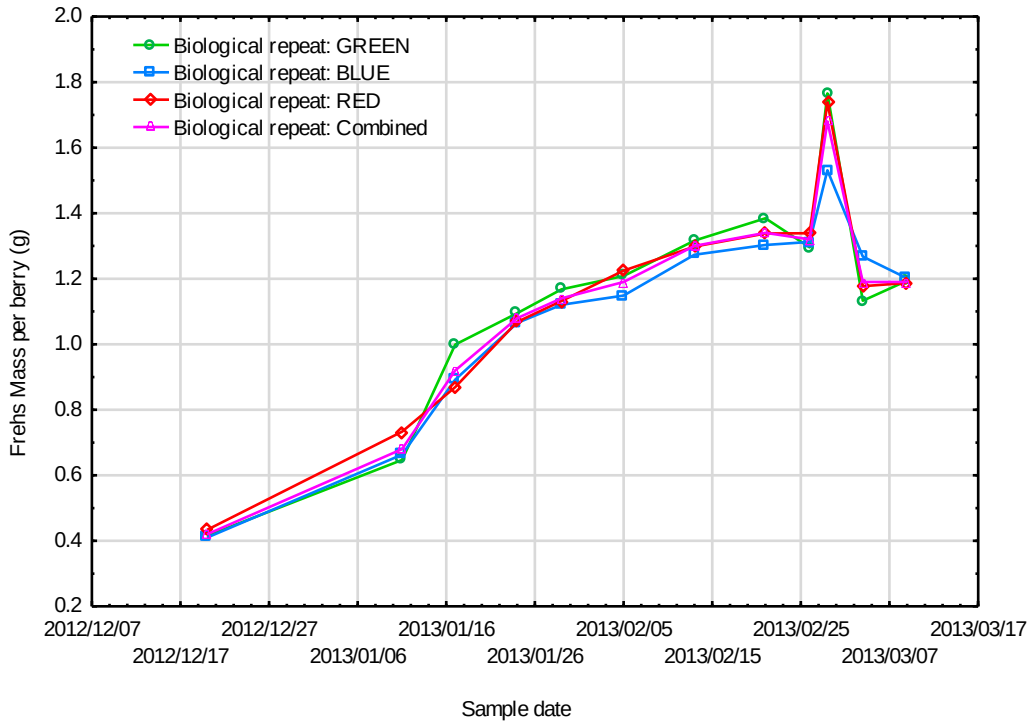

Figure 4.8: Fresh mass accumulation per berry per biological repeat

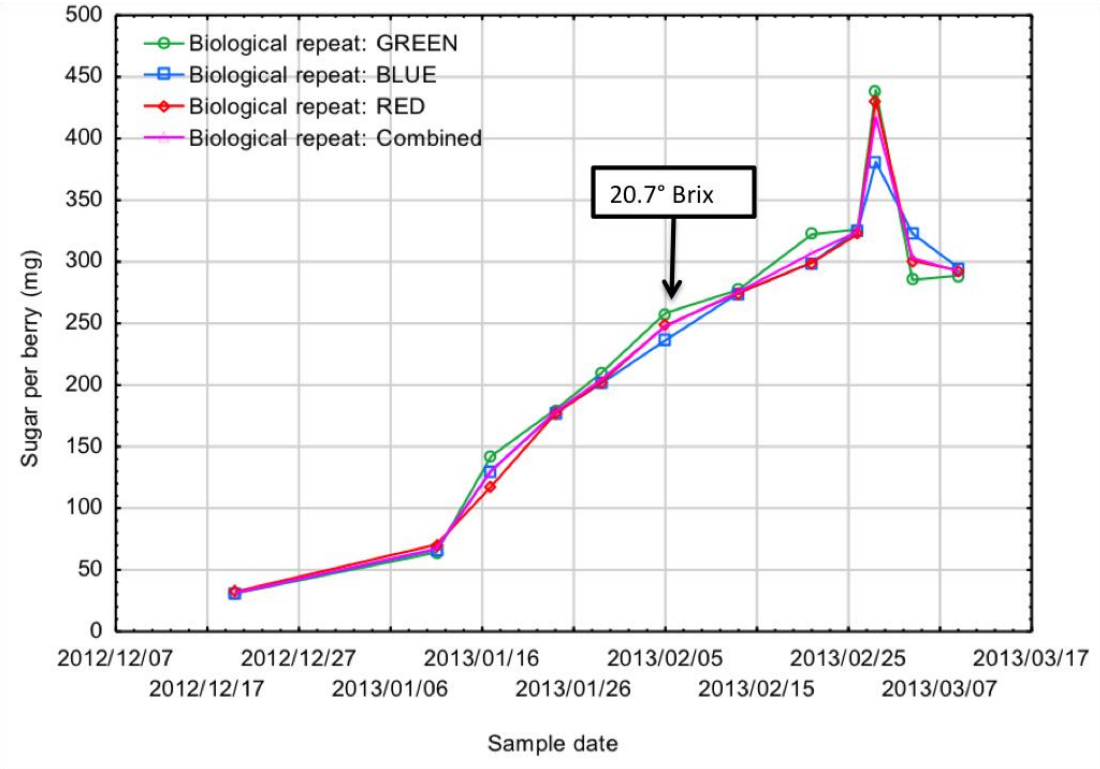

Figure 4.9: Sugar accumulation per berry (mg) per biological repeat

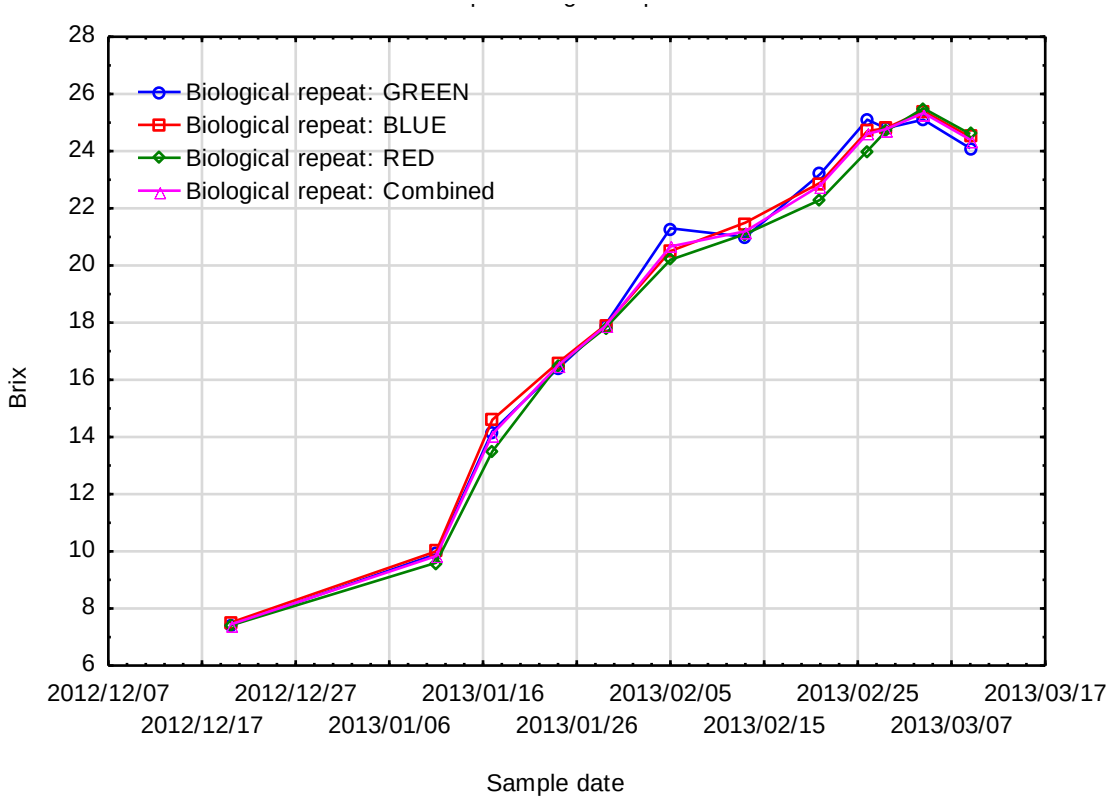

Figure 4.9: Brix level per biological repeat

It is important to have a homogenous plot when comparing wines and analysis as a difference in vigour can influence wine composition and quality (Hall *et al.,* 2002). For this reason, repeats are used as standard practice in research. This study paid careful attention to the layout of the 2013 plot after valuable experience gained in the layout of the 2012 project where strong differences in vigour were observed post *véraison*.

It is also important that the vine is in balance and the canopy characteristics are known for comparison with other studies or vintages. In this project, strong consideration was given to the homogeneity as far as possible within the vineyard block. Some detail may be overlooked when using a type of random block design on biological plots, as the small differences are averaged out. This can be seen when the data is displayed per panel, and the differences in vigour were highlighted by the NDVI.

The measurements of canopy characteristics and the NDVI showed that the plot could have been improved further by not selecting panels 1 and 9 as they differed the most in canopy measurements. However, for the scale of the experiment, the panels needed to be included and the heterogeneity was compensated for by using biological repeats. Using the NDVI alone would have sufficed in determining the plot. The disadvantage is the time it takes to process the hyperspectral images, however canopy measurements, such as leaf area, can only be done after the season as the methods are destructive.

4.3.5 Vine water status

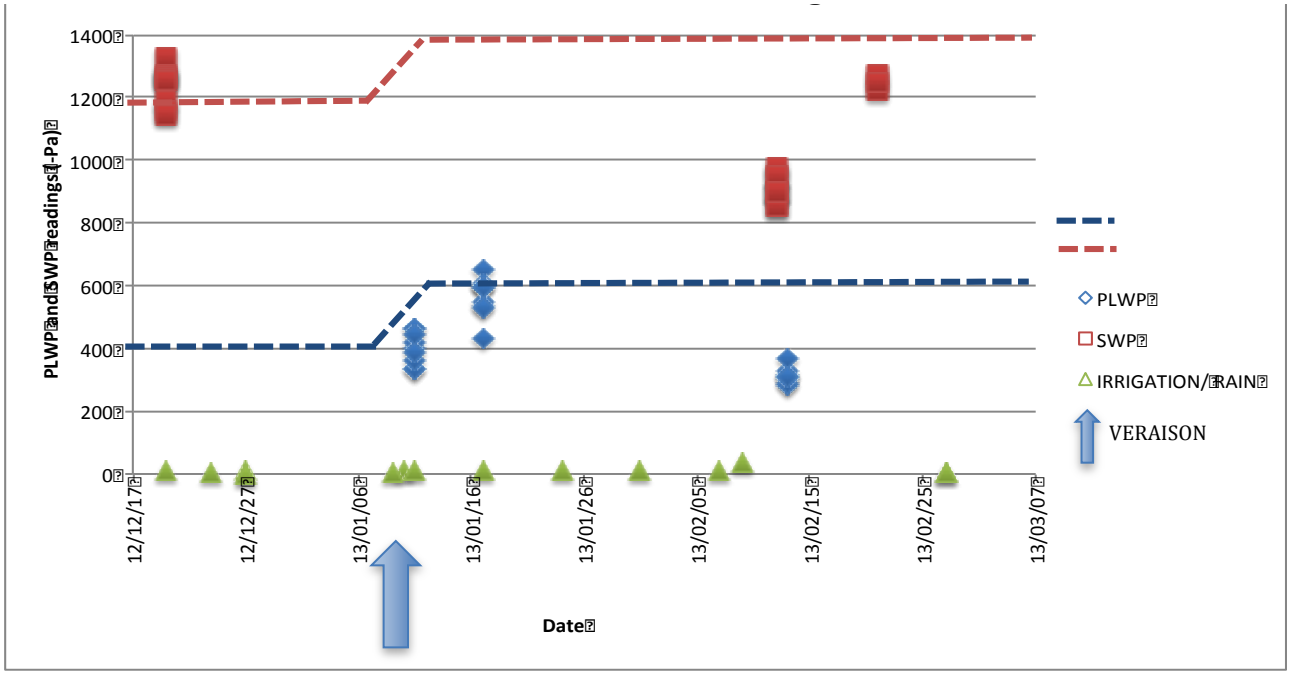

Figure 4.10 SWP and PLWP readings during 2013 season

Figure shows the rain and irrigation during the growing season and the PLWP and SWP indicate the aims for mild–moderate water constraints were reached. From Figure it can be seen that the irrigation was managed effectively as the SWP > -1200 kPa pre-*véraison* and <-1400 kPa post-*véraison* , with the exception of the first reading in early December which was followed by extensive irrigation. The PLWP was mostly within specification of PLWP >-400 kPa prior to *véraison* and >-600 kPa post-vérasion. On 17 January the average PLWP was -565 kPa, the red and blue biological repeats average were -600 kPa (see time point 2 in Figure) indicating that some vines experienced water constraints and irrigation was applied in real time to correct it.

SWP was also used to investigate homogeneity amongst the biological repeats. Analysis of variance was done on 54 SWP readings on 20 December 2012 and the differences between the red, blue and green biological repeats were not significant.

Similarly, the PLWP of the biological repeats was compared on 11 January 2013 and the differences between repeats were not statistically significant.

The mean PLWP for each biological repeat was compared over time (Figure) and although during the season the PLWP values changed, the differences between biological repeats means were not significant for each date considered. Time 3 showed water constraints, after which irrigation was applied.

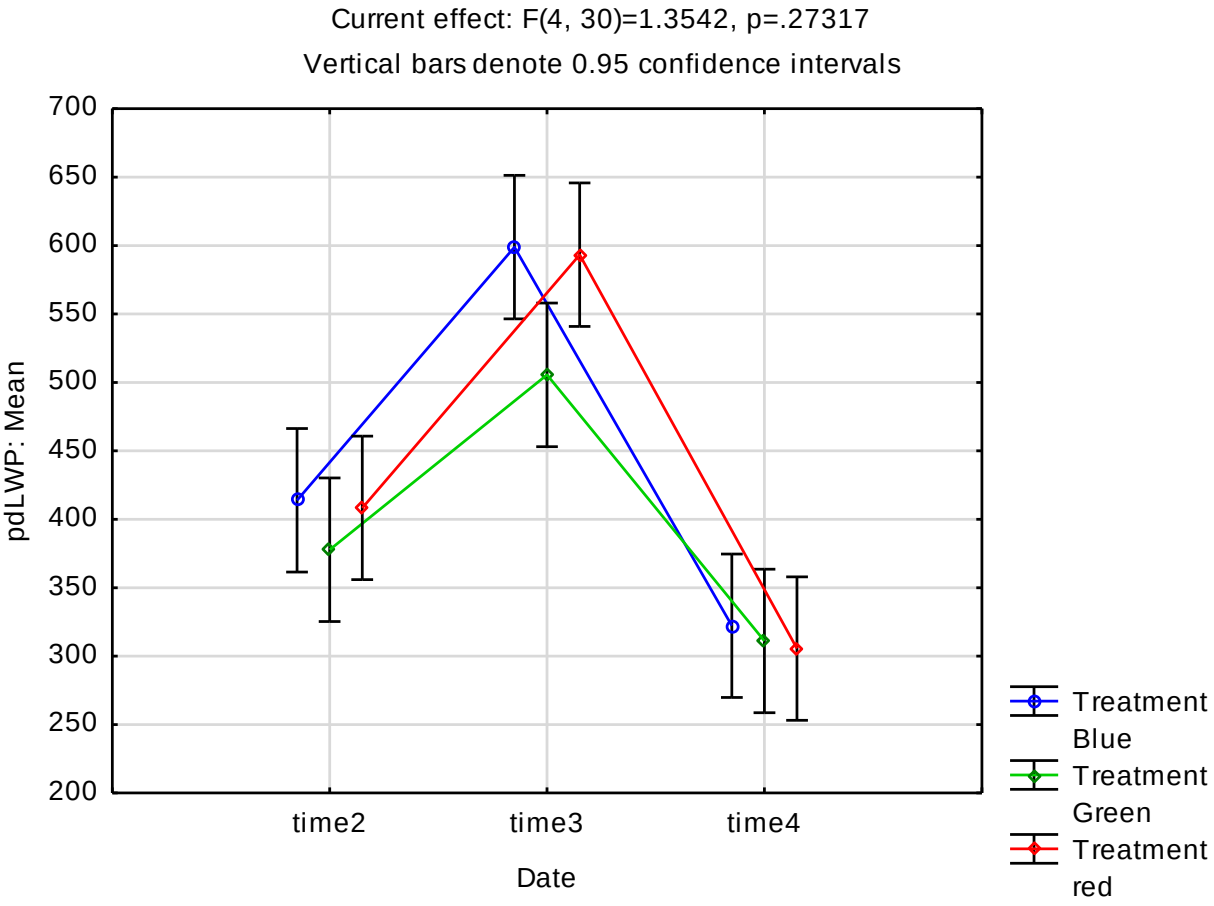

Figure 4.11: PLWP during the 2013 season (where time2 is 11 January 2013, time3 is 17 January 2013 and time4 is 13 February 2013).

4.3.6 Evolution of Durif grape composition

Sugar, acid, anthocyanin evolution

Evolution of sugar, acid and anthocyanin composition is linked to wine analysis and it is for this reason it has been included in the Chapter 5 in order to be discussed with the 2013 wine analysis.

4.4 Conclusion

The goal to use a homogenous vineyard site was achieved and supported by various results taken throughout the season and during winter pruning. This is particularly important in a study where berry composition and subsequent wine composition and aroma profiles are assessed. A detailed study was carried out including climatic data, NDVI imagery and vineyard measurements. The irrigation was managed such that the vines were subjected to moderate water constraints, some high water constraints were measured between 11 and 17 January 2013 by some of the vines and irrigation was applied.

4.5 Bibliography

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S. and Tabacman, H., 1985. Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. *American Journal of Enology and Viticulture,* 36(2), pp. 132-139.

Deloire, A. and Heyns, D., 2011. The leaf water potentials: Principles, method and thresholds. *Wineland,* 265, pp. 119-121.

Deloire, A., 2012. A few thoughts on grapevine training systems. *Wineland magazine,* 274, pp. 82-86.

Dokoozlian, N. and Kliewer, W., 1995. The light environment within grapevine canopies. Description and seasonal changes during fruit development. *American Journal of Enology and Viticulture,* 46(2), pp. 209-218.

Eichhorn, K. and Lorenz, D., 1977. Phenological development stages of the grape vine. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes,* 29(8), pp. 119-120.

Gregan, S., Wargent, J., LIU, L., Shinkle, J., Hofmann, R., Winefield, C., TROUGHT, M. and JORDAN, B., 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. *Australian Journal of Grape and Wine Research,* 18(2), pp. 227-238.

Hall, A., Lamb, D., Holzapfel, B. and Louis, J., 2002. Optical remote sensing applications in viticulture: a review. *Australian Journal of Grape and Wine Research,* 8(1), pp. 36-47.

Huglin, P., 1978. Presentation internationale uniforme des resultats d'essais viticoles [teneur en sucre des mouts, acidite totale, rendement au vignoble, donnees bioclimatiques]. *Bulletin de l'OIV,* .

Johnson, L., Roczen, D., Youkhana, S., Nemani, R. and Bosch, D., 2003. Mapping vineyard leaf area with multispectral satellite imagery. *Computers and Electronics in Agriculture,* 38(1), pp. 33-44.

Kliewer, W.M. and Dokoozlian, N.K., 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *American Journal of Enology and Viticulture,* 56(2), pp. 170-181.

Parker, A., Hofmann, R., Leeuwen, C., Mclachlan, A. and Trought, M., 2015. Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable acidity of grape berries. *Australian Journal of Grape and Wine Research,* pp. 266-276.

Scheiner, J.J., Sacks, G.L., Pan, B., Ennahli, S., Tarlton, L., Wise, A., Lerch, S.D. and Heuvel, J.E.V., 2010. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red wine grapes. *American Journal of Enology and Viticulture,* 61(3), pp. 358-364.

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A. and Hammel, H.T., 1965. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. *Science (New York, N.Y.),* 148 (3668), pp. 339-346.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. and Qian, M.C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. *Food Chemistry,* 173, pp. 424-431.

Šuklje, K., Česnik, H.B., Janeš, L., Kmecl, V., Vanzo, A., Deloire, A., Sivilotti, P. and Lisjak, K., 2013. The effect of leaf area to yield ratio on secondary metabolites in grapes and wines of Vitis vinifera L. cv. Sauvignon blanc. *Journal International des Sciences de la Vigne et du Vin,* 47, pp. 83-97.

Tonietto, J. and Carbonneau, A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology,* 124(1), pp. 81-97.

Wessner, L.F. and Kurtural, S.K., 2012. Pruning systems and canopy management practice interact on the yield, and fruit composition of Syrah. *American Journal of Enology and Viticul*ture, 64.1 pp. 134-138.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A. 1974. *General Viticulture* (2nd edition). University of California Press.

Young, P.R., Lashbrooke, J.G., Alexandersson, E., Jacobson, D., Moser, C., Velasco, R. and Vivier, M.A., 2012. The genes and enzymes of the carotenoid metabolic pathway in Vitis vinifera L. *BMC genomics,* 13, pp. 243-2164-13-243.

Chapter 5

Using sequential harvesting to assess Durif wine styles using berry sugar loading as a physiological indicator

Chapter 5: Using sequential harvesting to assess Durif wine styles using berry sugar loading as a physiological indicator

5.1 Introduction

The goal of this research project was to evaluate the potential wine profiles that can be achieved from Durif grapes using sequential harvesting. This includes defining the harvest timing using berry physiological indicators to identify fresh fruit and mature fruit style wines. The objective is to harvest at different stages of ripeness (on different dates). A physiological indicator, berry sugar loading, was used to determine harvest dates. Grapes were harvested from a range of 22 to 26 degrees Brix. Sensory analysis was used to determine the wine sensory attributes of the ripening stages (made from sequentially harvested grapes) corresponding to fresh fruit and mature fruit style wines. The goal of the sensory assessment is to determine the main aromatic attributes associated with Durif wine in general and with fresh and mature fruit profile in particular. This was achieved with the help of the frequency of citation method. Additionally, the mouthfeel of Durif wines was investigated and how sequential harvest can affect the perception of tannin in Durif wines was evaluated. This was achieved by using descriptive analysis for the mouthfeel perceptions during sensory analysis.

5.2 Materials and methods

5.2.1 Monitoring berry sugar accumulation and the tempo of ripening

From *véraison* the berry mass evolution and Brix were monitored together with other indicators, namely pH and TA. Grapes were frozen for later analysis in order to study the evolution of tartaric and malic acid, fructose and glucose and anthocyanins. The methods for these analyses are described in Chapter 3 (Determining fruit composition). The Brix and berry fresh mass were used to calculate the sugar accumulation per berry and sugar concentration using the principles of sugar loading (SL) (Deloire, 2011). The average Brix and berry fresh mass of the three biological repeats were used to determine when sugar loading stopped (key point) and sequential harvests were planned according to this physiological date using the model developed for Syrah, assessing that the tempo of ripening will be very similar for Durif (Šuklje *et al.,* 2014).

Fresh and dry mass

Fresh mass was determined by weighing 50 berries. Dry mass of the berries was determined by weighing 50 berries after allowing the berries to dry in an oven for a minimum 72 hours at 60 °C. This is a destructive method; therefore the data set is different to fresh mass data used to calculate SL.

5.2.2 Sequential harvest

Harvest dates were predetermined using the SL model. Five dates were selected for sequential harvest and one wine was made per biological repeat for each date (three wines per date). Vines were selected randomly within the plot and only one vine per panel was selected for each harvest. The entire vine was harvested to avoid altering the physiological functioning of the berries by crop thinning (in order to reduce the risk of sampling or harvesting from this vine which has had its crop thinned). Each vine's yield, number of bunches and average bunch mass was determined. The predetermined or planned dates had a window period for harvesting (approximately three days after the determined date was still considered acceptable) to take into account climatic factors. For example, the harvest on 01/03/2013 was scheduled earlier but due to rain it was moved two days later.

5.2.3 Winemaking

The wine was made at the experimental cellar of the University of Stellenbosch, South Africa as described in Chapter 3 (3.2.7).

5.2.4 Determining wine composition

Analyses including pH, TA, alcohol, volatile acidity and residual sugar were performed. Tannins (BSA precipitation method (Hagerman and Butler, 1978)), total red pigment colour, wine colour density, wine colour hue and degree of red pigment colouration (South African Wine Laboratories Association (SAWLA), 2002) were determined. The methods are described in Chapter 3 (3.2.9)

5.2.5 Sensory analysis of sequentially harvested wines

The frequency of citation method (Campo *et al.,* 2010) was used for odour description and intensity scores were used for mouthfeel ratings. The methods are described in Chapter 3. Random codes were allocated to the wines and the following abbreviations were used for the different biological repeats and harvest dates.

Table 1: Abbreviations used for each harvest date and biological repeat

Harvest date	Harvest number	Biological repeat	Code
2013/02/22	Harvest 1	Blue	H1_B
2013/02/22	Harvest 1	Green	H1_G
2013/02/22	Harvest 1	Red	H1_R
2013/02/25	Harvest 2	Blue	H2_B
2013/02/25	Harvest 2	Green	H2_G
2013/02/25	Harvest 2	Red	H2_R
2013/03/01	Harvest 3	Blue	H3_B
2013/03/01	Harvest 3	Green	H3_G
2013/03/01	Harvest 3	Red	H3_R
2013/03/04	Harvest 4	Blue	H4_B
2013/03/04	Harvest 4	Green	H4_G
2013/03/04	Harvest 4	Red	H4_R
2013/03/08	Harvest 5	Blue	H5_B
2013/03/08	Harvest 5	Green	H5_G
2013/03/08	Harvest 5	Red	H5_R

5.2.6 Statistical analysis

General statistical analysis included Analysis Of Variance (ANOVA). Mean comparisons were performed using Fisher's least significant difference (LSD) test ($p \leq 0.05$). Mean comparisons with confidence intervals set at 0.95 were used to show trends (Software: Statistica), where letters indicate significant difference ($p < 0.5$).

5.3 Results and Discussion

5.3.1 Grape analysis

Berry sugar accumulation, sugar loading curves and determination of the ripening stages

The SL model was used to determine the keypoint. Plateaus of sugar and berry fresh mass were reached as per the model curve (Deloire, 2011). The increase in both sugar per berry and fresh mass was due to late season rainfall, triggering a brief 'reloading' of sugar, due to enhancement of photosynthesis or sugar remobilization from leaves or roots - this stabilized soon after (Figure 5.1, Figure 5.2). The keypoint was established as 13 February 2013, when the rate of sugar accumulation slowed to 3.50 mg/day (Deloire, 2013). This could be confirmed retrospectively (Table 2) as the Brix was close to 20 (approximate estimated range 18–20 Brix) and berry volume is 77% of the total – both indicators of reaching the plateau.

Table 2: The evolution of Brix, fresh mass, sugar per berry and sugar per berry per day throughout the ripening period

Growing Degree Days (GDD)	Date	Brix *(Standard error =0.2)*	Fresh mass per berry (g/berry) *(Standard error = 0.03)*	Sugar per berry (mg) *(Standard error = 7.17)*	Sugar per berry per day (mg/day)
291.76	2012/12/20	7.4^{j}	0.42^{g}	$31,28^{j}$	
561.91	2013/01/11	9.8^{i}	0.68^{f}	$67,15^{i}$	1,63
655.23	2013/01/17	14.1^{h}	0.92^{e}	$130,16^{h}$	10,50
755.46	2013/01/24	16.5^{g}	1.08^{d}	$178,23^{g}$	6,87
830.67	2013/01/29	17.9^{f}	1.14^{cd}	$204,33^{f}$	5,22
935.88	2013/02/05	20.7^{e}	1.19^{c}	$247,57^{e}$	6,18
1049.06	**2013/02/13**	**21.2^{d}**	**1.30^{b}**	**$275,58^{d}$**	**3,50**
1140.52	2013/02/21	22.8^{c}	1.34^{b}	$306,86^{bc}$	3,91
1201.46	2013/02/26	24.6^{b}	1.32^{b}	$324,60^{b}$	3,55
1223.45	2013/02/28	24.8^{b}	1.68^{a}	$416,94^{a}$	46,17
1285.52	2013/03/04	25.3^{a}	1.19^{c}	$303,00^{c}$	-28,49
1336.35	2013/03/09	24.4^{b}	1.19^{c}	$292,26^{cd}$	-2,15

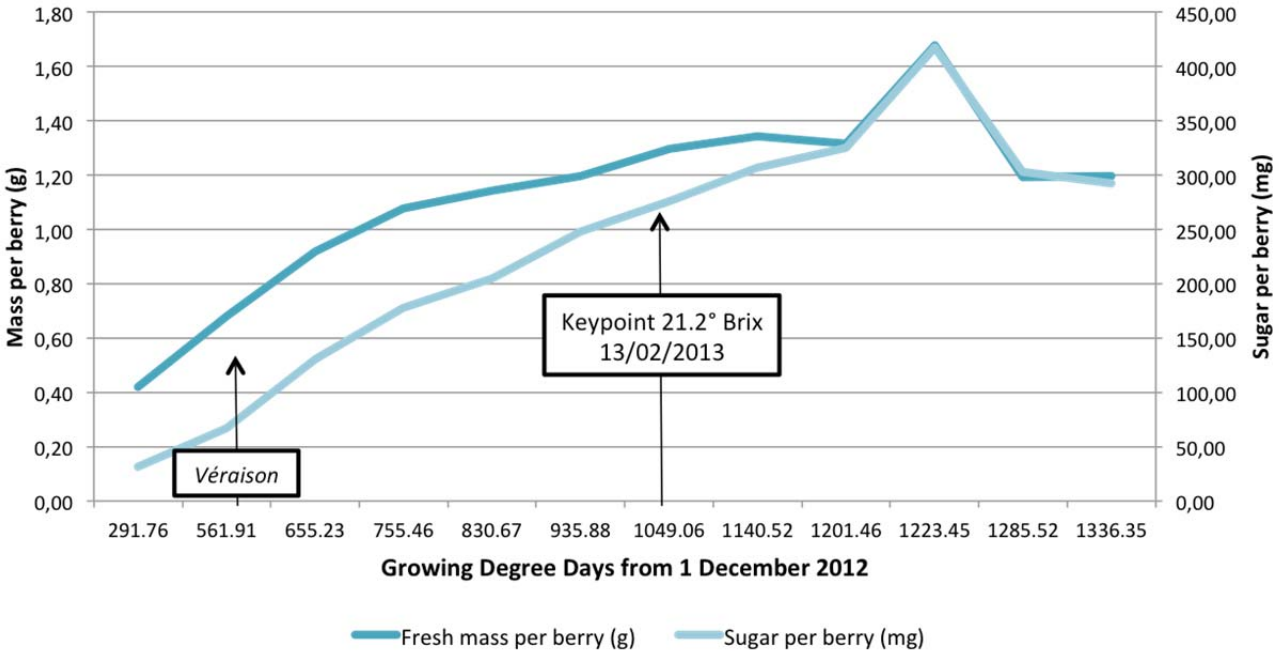

Figure 5.1: Sugar and fresh mass accumulation per berry with reference to GDD

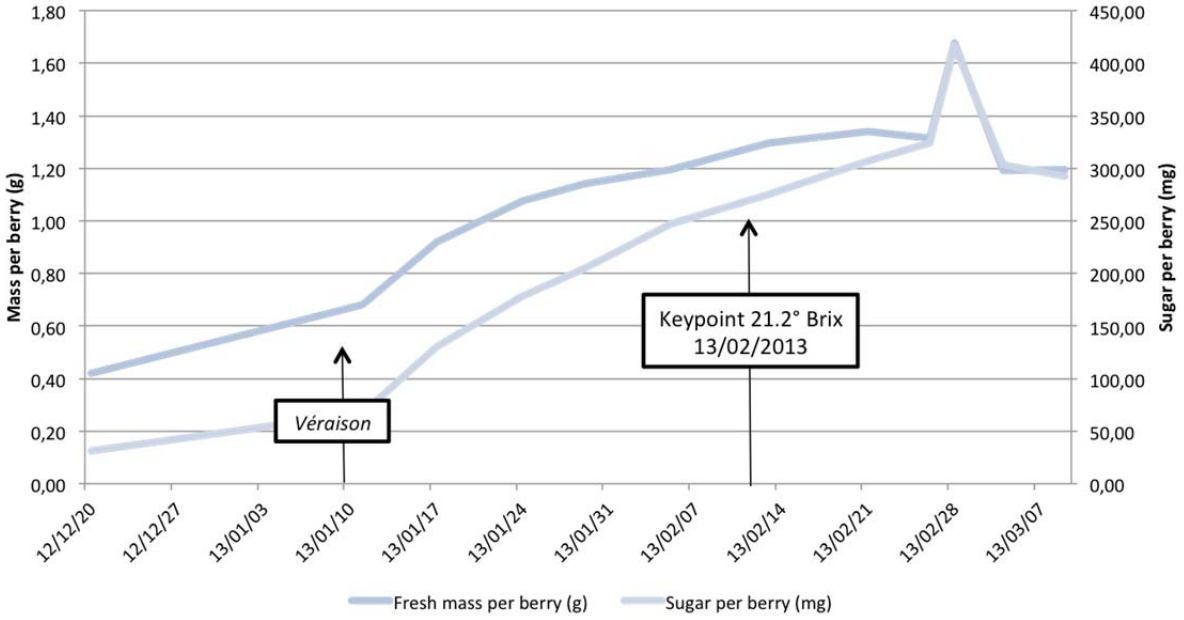

Figure 5.2: Sugar and fresh mass accumulation per berry with reference to calendar dates

The Brix accumulation is typical, showing rapid accumulation post véraison and a decrease in rate towards ripening (Figure 5.3). Berry fresh mass increased rapidly post véraison (Figure 5.4). After the keypoint, 13 February 2013, the fresh mass remained stable – increasing sharply on 28 February 2013 due to rainfall and decreasing thereafter. The consistent fresh mass curve indicates there was no berry shriveling during ripening or severe water stress, which can lead to 20–30% decrease in berry volume in warm conditions. The sugar per berry continued to increase (Figure 5.4) with the increasing fresh mass and Brix. The rate of increase in sugar per berry decreased from 17 January 2013 (10.50 mg per berry per day) and decreases further between 5 and 13

February 2013 to 3.5 mg per berry per day. There was a rapid reloading and increase to 46.17 mg per berry per day on the 28th February due to rainfall, increasing both sugar per berry and volume.

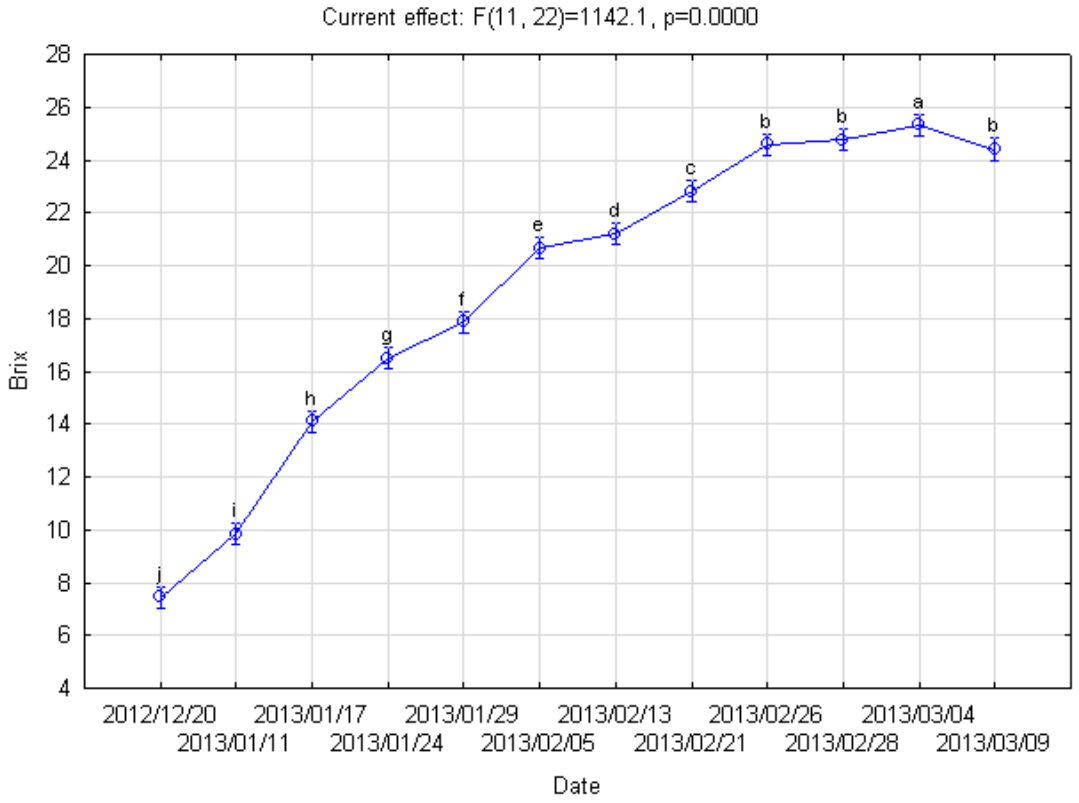

Figure 5.3: Evolution of Brix

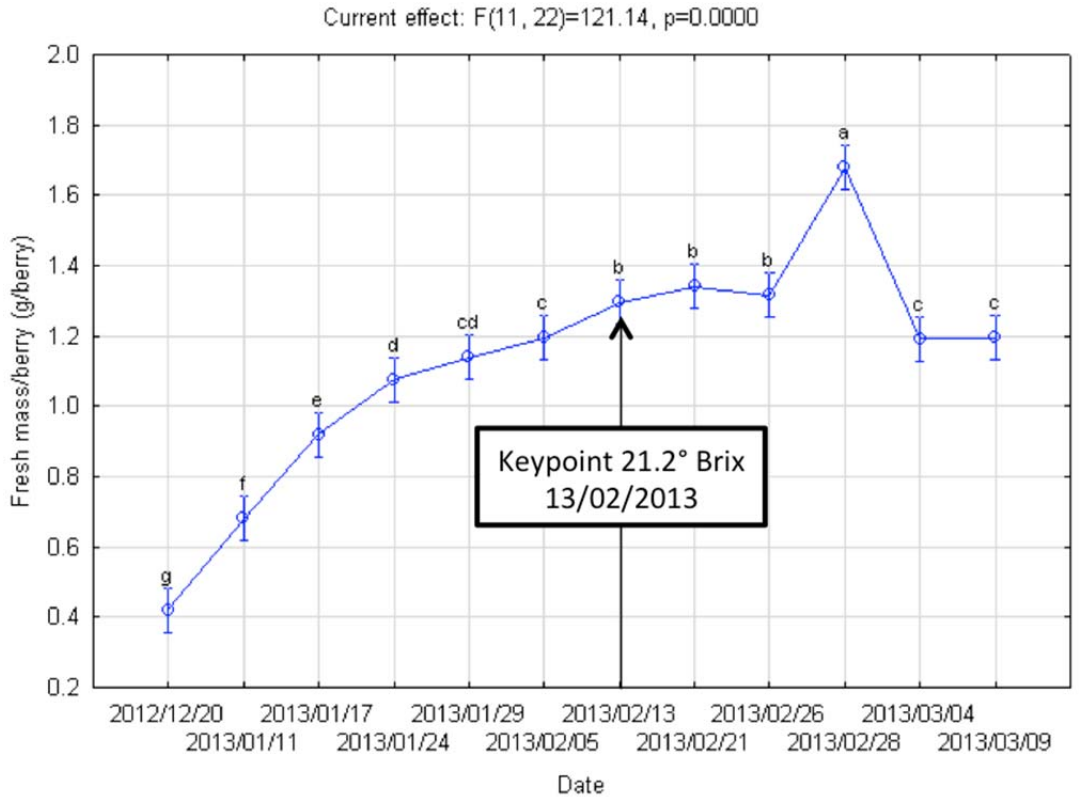

Figure 5.4: Evolution of berry fresh mass

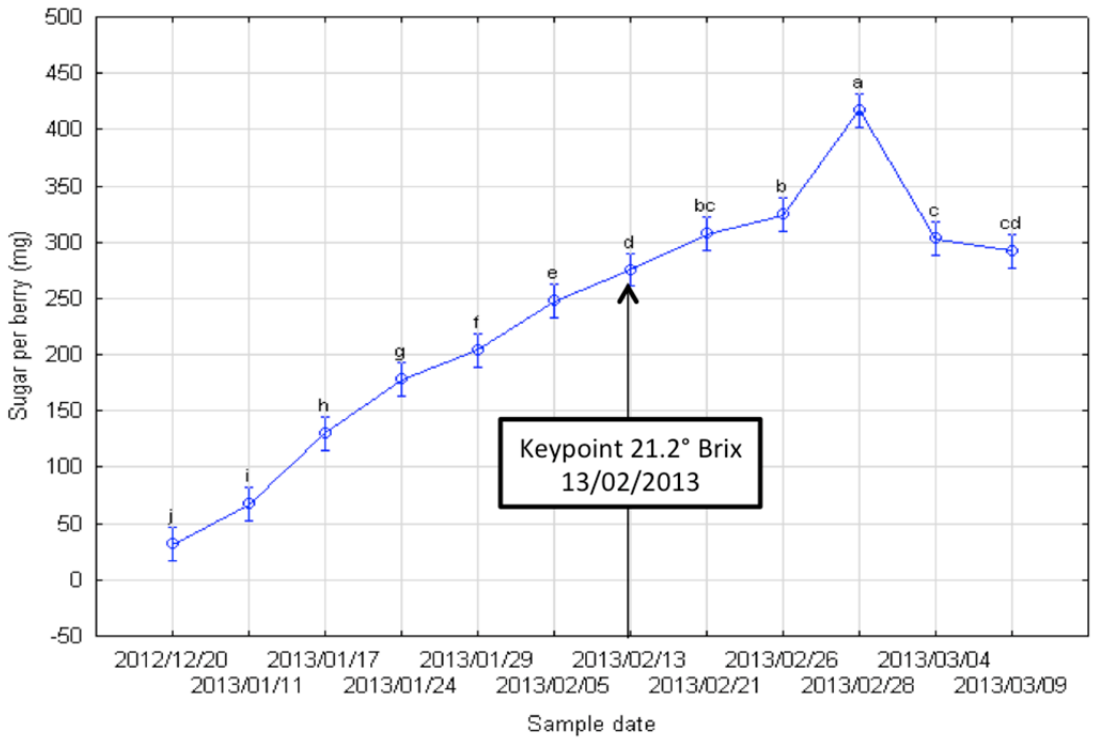

Figure 5.4: Sugar accumulation per berry

Sequential harvest and winemaking

The keypoint was established as 13 February 2013. The harvest dates were selected based on the norms for fresh fruit profile wines for most varieties, 10–12 days after the keypoint (Deloire, 2011). For mature fruit, 15, 20 and 25 days after the keypoint were considered, in order to calibrate the model using the information from Shiraz, Merlot and Cabernet Sauvignon (Deloire, 2011; Deloire, 2013). Taking into account the weather, increasing Brix and practicalities around harvesting, the dates shown in Table 3 were selected for sequential harvest.

Table 3: Summary of harvest dates and days from keypoint

Harvest number	Harvest date	Days from keypoint (13 February 2013)
1	22/02/2013	9
2	25/02/2013	12
3	01/03/2013	16
4	04/03/2013	19
5	08/03/2013	23

Berry fresh and dry mass accumulation

The fresh and dry mass measurements ran concurrently to sugar loading measurements and unfortunately did not capture the peak in FM on the 28 February 2013 (1.68 gram per berry) after rainfall. From 24 January 2013, the rate of fresh mass accumulation decreased (Figure 5.5). 77% dry mass (0.27g/berry) was reached on 24 January 2013. There were no significant differences in dry mass between 24 and 29 January 2013 (Figure 5.6). The dry mass remained constant after 13 February 2013, when keypoint was reached.

Table 4: Fresh and dry mass per berry

Date	Fresh mass per berry (g/berry) *Standard Error = 0.043*	Dry Mass per berry (g/berry) *Standard Error = 0.01*	Percent of water per berry (%)	Difference between Fresh Mass and Dry Mass per berry (g/berry) *Standard Error = 0.033*
2013/01/11	0.783^{d}	0.138^{e}	82%	0.645^{d}
2013/01/17	0.953^{c}	0.195^{d}	80%	0.758^{c}
2013/01/24	1.230^{b}	0.273^{c}	78%	0.957^{ab}
2013/01/29	1.203^{b}	0.267^{c}	78%	0.937^{b}
2013/02/05	1.269^{b}	0.324^{b}	74%	0.945^{ab}
2013/02/13	1.384^{a}	0.357^{a}	74%	1.027^{a}
2013/02/26	1.186^{b}	0.351^{ab}	70%	0.835^{c}

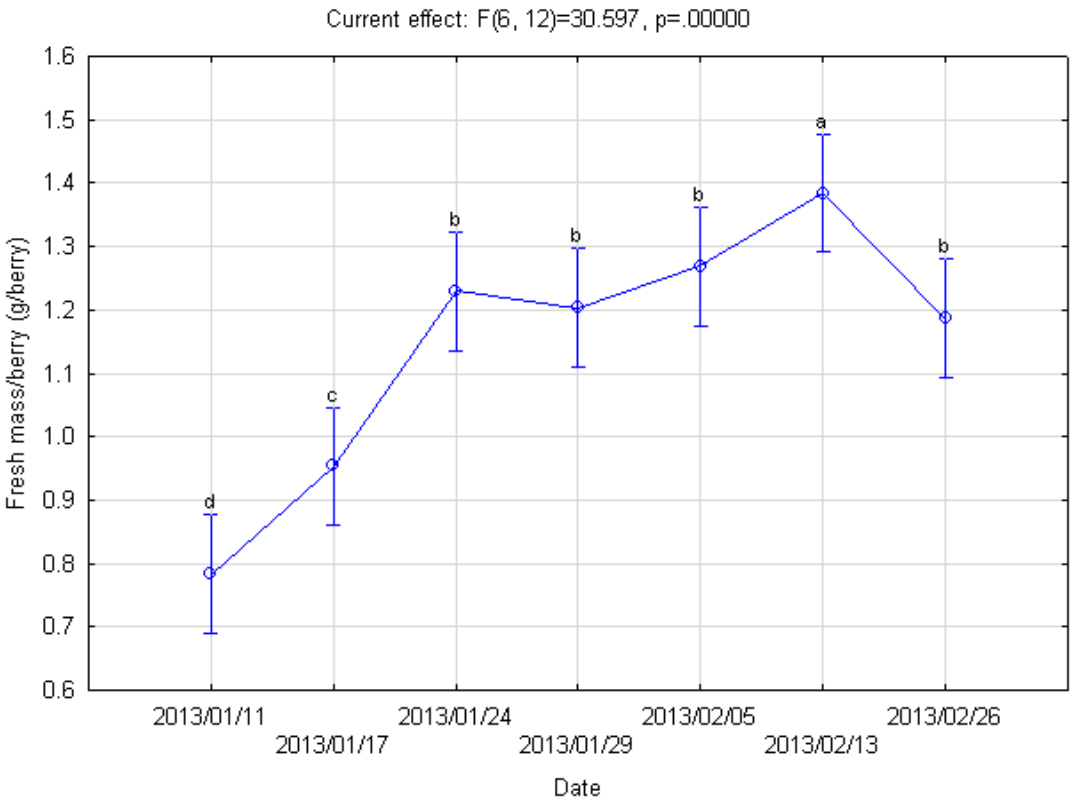

Figure 5.5: Evolution of berry fresh mass (gram per berry)

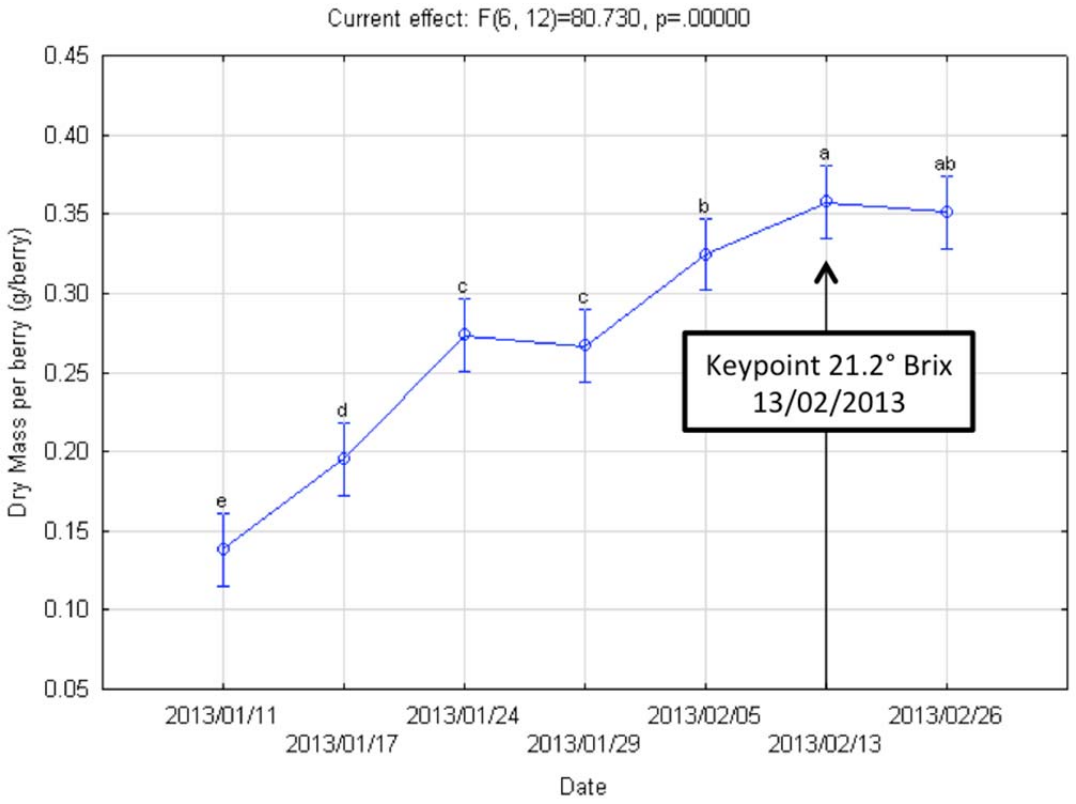

Figure 5.6: Evolution of berry dry mass (gram per berry)

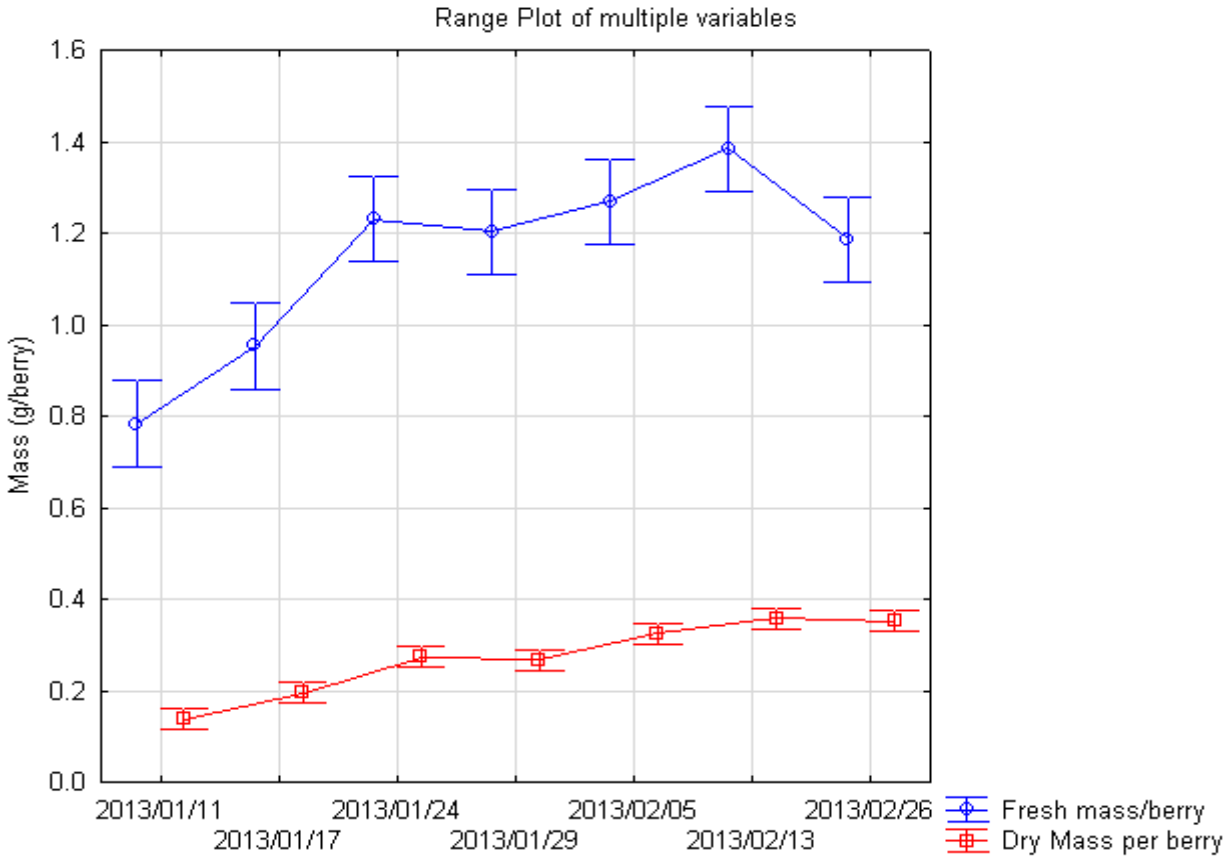

Figure 5.7: Plot of fresh mass and dry mass evolution per berry

Fructose and glucose accumulation

As expected, the glucose and fructose per berry and per gram berry increased during ripening (see Figure B1, B2, B3, B4 in Appendix B.2 and Table 5, below) (Conde *et al.,* 2007) and the glucose to fructose ratio decreased to below 1 post-véraison (11 January 2013 was a pre-vérasion sample) (Ribéreau-Gayon *et al.,* 2000). The rate of fructose and glucose (as well as fresh and dry mass) accumulation decreased from 24 January 2013. Glucose and fructose continued to increase to maximum value on 26 and 28 February 2013 when there was a spike in the berry mass due to rainfall (Figure B1, B2, B3, B4 in Appendix B.2). The sum of glucose and fructose corresponds to the SL model and confirms the keypoint as 13 February 2013.

Table 5: Fructose and glucose per berry and per gram berry

Sample date	D-Fructose per gram berry	D-Fructose per berry	D-Glucose per gram berry	D-Glucose per berry	Mean glucose: fructose
13/01/11	20.1^{f}	14.31^{f}	24.76^{f}	18.05^{g}	1.158
13/01/17	45.14^{e}	48.52^{e}	44.21^{e}	45.15^{f}	0.977
13/01/24	61.26^{d}	77.78^{d}	59.05^{d}	76.58^{e}	0.968
13/01/29	66.98^{cd}	83.93^{dc}	67.38^{d}	79.56^{e}	0.952
13/02/05	79.44^{cb}	108.68^{bc}	68.86^{d}	88.5^{ed}	0.931
13/02/13	76.66^{cd}	114.88^{b}	67.65^{d}	101.56^{cd}	0.933
13/02/21	93.1^{ab}	132.93^{ab}	84.01^{c}	115.98^{cb}	0.923
13/02/26	93.88^{ab}	155.32^{a}	86,59^{cb}	135.21^{ab}	0.936
13/02/28	103.94^{a}	146.91^{a}	98.16^{a}	137.16^{a}	0.918
13/03/04	104.45^{a}	13522^{ab}	97.28^{ab}	121.73^{ac}	0.907
13/03/09	103.48^{a}	130.88^{ab}	97.12^{ab}	125.76^{ab}	0.938

Assessment of the sugar loading model

A strong correlation (R = 0.91) was found between the sum of D-fructose and D-glucose (mg per berry) (Table 5) and the calculated sugar per berry (mg) (Table 2). This confirms that, in this case, the equation used to estimate the sugar per berry is accurate, even though it is considered an estimation depending on seed volume and distribution of sugar in the pulp (Deloire, 2011), and can be used during harvest time when time-consuming laboratory analysis is not necessarily practical in making harvest decisions.

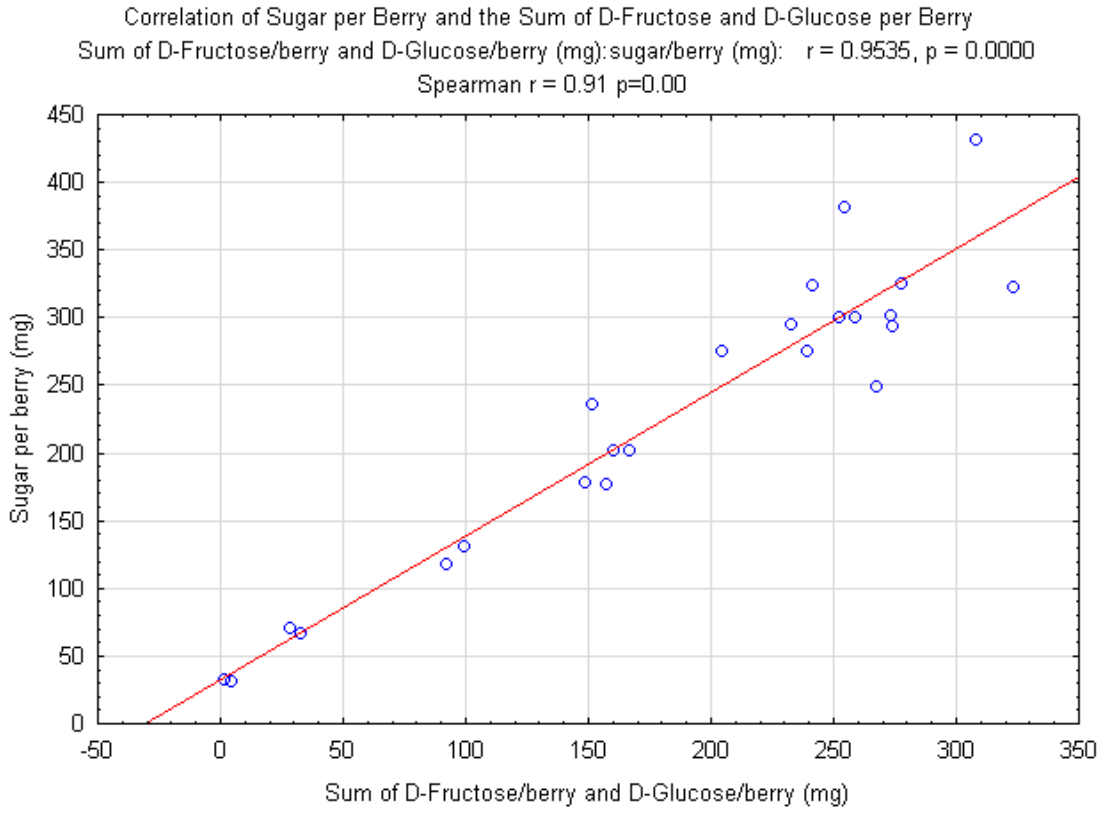

Figure 5.8: Correlation of the sum of fructose and glucose to the calculated sugar/berry (2013)

On comparing the sugar per berry graph (Figure 5.2) to the glucose and fructose per berry graphs (Figure 5.5 and Appendix B.2 Figure B1 and B2) it seems the sugar loading model overestimated the contribution of the increase in berry volume on 26 February 2013 as the sum of glucose and fructose on this date was equal to 290 mg (Table 5) versus the (over) estimated 324 mg (Table 2), and on 28 February was equal to 284 mg (Table 5) versus the (over) estimated 416 mg (Table 2).

Degradation of organic acids

Malic acid (mg/berry) increased (Figure B5 and B6 in Appendix B.2) significantly as the berry size increased prior to véraison, while concentration (mg/g berry) shows no significant increase prior to véraison as the rate of accumulation is proportional to the increase in berry size (Table 6). Towards ripening, the berry size is more stable and changes in concentration are more noticeable. Accumulation of malic acid reaches its peak prior to véraison (Conde *et al.,* 2007) after which there is a sudden rapid decrease in malic acid concentration as expected (Coombe, 1992). This is due to catabolism and a decrease in translocation as summarized in a review by Conde *et al.,* (2007). After 5 February 2013 there was no significant change in malic acid per berry or per gram berry, possibly due to a balance between catabolism and anabolism (Conde *et al.,* 2007).

Tartaric acid (mg/berry) decreased (Figure B7 and B8 in Appendix B) due to an initial increase in berry volume and tartaric acid degradation, as can be expected (Conde *et al.,* 2007) (Table 6). The rate of tartaric acid degradation decreased from 24 January 2013 and the decrease in tartaric acid concentration (mg/g berry) is less significant with small fluctuations as the berry volume stabilizes. The differences in tartaric acid per berry after 24 January 2013 are not significant (Table 6).

Table 6: Evolution of Tartaric and L-Malic acid

Sample date	Fresh mass (g/berry)	L-Malic acid (mg/g berry)	L-Malic acid (mg/berry)	Tartaric acid (mg/g berry)	Tartaric acid (mg/berry)
12/12/20	0.42^{g}	10.77^{a}	4.13^{c}	17.13^{a}	6.50^{ab}
13/01/11	0.68^{f}	10.28^{a}	7.47^{a}	9.32^{b}	6.77^{ab}
13/01/17	0.92^{e}	5.34^{b}	5.46^{b}	7.41^{c}	7.57^{a}
13/01/24	1.08^{d}	2.87^{c}	3.71^{c}	5.38^{d}	6.95^{ab}
13/01/29	1.14^{cd}	2.12^{d}	2.55^{d}	4.87^{de}	5.83^{b}
13/02/05	1.19^{c}	1.28^{e}	1.66^{e}	5.33^{d}	6.73^{ab}
13/02/13	1.30^{b}	0.94^{e}	1.4^{e}	3.79^{e}	5.71^{b}
13/02/21	1.34^{b}	1.16^{e}	1.63^{e}	4.74^{de}	6.53^{ab}
13/02/26	1.32^{b}	1.05^{e}	1.63^{e}	3.63^{e}	5.75^{b}
13/02/28	1.68^{a}	0.92^{e}	1.29^{e}	4.6^{de}	6.38^{ab}
13/03/04	1.19^{c}	1.25^{e}	1.57^{e}	5.28^{d}	6.69^{ab}
13/03/09	1.19^{c}	1^{e}	1.31^{e}	4.86^{de}	6.27^{ab}

Accumulation of total anthocyanins and total phenolics

Phenolics represent 90–95% of compounds absorbing light at 280 nm in grapes (Pirie and Mullins, 1977). Total phenolics are expected to peak prior to véraison, thereafter there is a decrease in phenolics concentration with a general trend towards lower phenolics in later harvests (Singleton, 1966). In the data, a decline in absorbance per gram berry can be seen after 20 December 2012 due to an increase in berry volume. Thereafter, the absorbance per gram berry remained relatively stable, increasing at the same rate as berry volume. The absorbance per berry peaks on 5 February 2013, at the same timing as the peak in anthocyanins, and then remains relatively stable during ripening (Table 7 and Figure B9 and B10 in Appendix B.2).

Anthocyanin accumulation per berry peaked on 5 February 2013 (Table 7 and Figure B11 and B12 in Appendix B.2), and stabilized thereafter. Anthocyanin absorbance per gram of berry also increases, as expected, post-véraison at a greater rate than the increasing berry volume. The rate of anthocyanin per gram berry accumulation decreased after 5 February 2013 as the berry volume stabilized, and the concentration continued to increase at a slower rate. The concentration per gram of berry peaked on 28 February 2013 (Figure 5.9) when berry volume was at its maximum. The trend observed for anthocyanin accumulation to peak followed by a slight decrease and then increase to another peak is similar to the findings in Tempranillo and Cabernet Sauvignon (Ryan and Revilla, 2003).

Bindon *et al.,* (2013) monitored grape anthocyanins and tannins for five sequential harvests. They found that anthocyanins increased per berry and per gram berry with increasing ripeness. On comparing the anthocyanin concentration for Durif from 22 February 2013 (H1) to 8 March 2013 (H5), there were no significant differences in the anthocyanin per berry, there was a small increase on 28 February 2013 and then there was a net decrease. As suggested by Ribereau Gayon *et al.,* (2000) the maximum level of anthocyanins is at full ripeness followed by a degradation as the grapes becomes overripe. The trends for anthocyanins per gram of berry were similar, the only significant difference was between 26 and 28 February 2013, which is most likely due to berry volume. The net increase in anthocyanins per gram berry over this period is in agreement with the findings of Bindon *et al.,* (2013). Total phenolics per gram berry showed a net decrease, which is in agreement with the findings of Bindon *et al.,* (2013).

Table 7: The evolution of Total Phenolics and Total Anthocyanins, measured in absorption at 280 and 520 nm, respectively

Sample date	Fresh mass (mg/berry)	Abs 280 nm (AU/g berry)	Abs 280 nm (AU/berry)	Abs 520 nm (AU/g berry)	Abs 520 nm (AU/ berry)
12/12/20	0.42^{g}	490.99^{a}	4.414^{ab}	6.25^{e}	0.056^{f}
13/01/11	0.68^{f}	128.02^{b}	2.283^{d}	4.81^{e}	0.085^{f}
13/01/17	0.92^{e}	102.71^{bc}	2.414^{d}	18.31^{e}	0.42^{fe}
13/01/24	1.08^{d}	112.7^{bc}	3.173^{db}	35.88^{d}	1.01^{de}
13/01/29	1.14^{cd}	105.99^{bc}	3.191^{db}	44.28^{dc}	1.34^{dc}
13/02/05	1.19^{c}	104.96^{bc}	4.598^{a}	53.25^{abc}	2.37^{a}
13/02/13	1.30^{b}	81.12^{c}	2.862^{dc}	43.08^{dc}	1.51^{db}
13/02/21	1.34^{b}	199.05^{bc}	3.213^{db}	57.64^{abc}	1.84^{abc}
13/02/26	1.32^{b}	86.85^{c}	3.175^{db}	49.94^{db}	1.82^{abc}
13/02/28	1.68^{a}	119.73^{bc}	3.886^{abc}	65.65^{a}	2.12^{ab}
13/03/04	1.19^{c}	96.12^{bc}	3.003^{dc}	53.21^{abc}	1.66^{ad}
13/03/09	1.19^{c}	106.26^{bc}	3.143^{db}	59.78^{ab}	1.76^{ad}

Figure 5.10 shows the rapid accumulation of anthocyanins, sugars and fresh mass from véraison onwards. Anthocyanin accumulation coincides with the onset of rapid sugar loading (Figure 5.9), corresponding to the literature (Singleton, 1966). The onset of rapid anthocyanin (per berry) accumulation was approximately one week (significant increase in anthocyanin per berry on 24 January 2013) after the onset of sugar accumulation, similar to the findings of Pirie and Mullins, (1977).

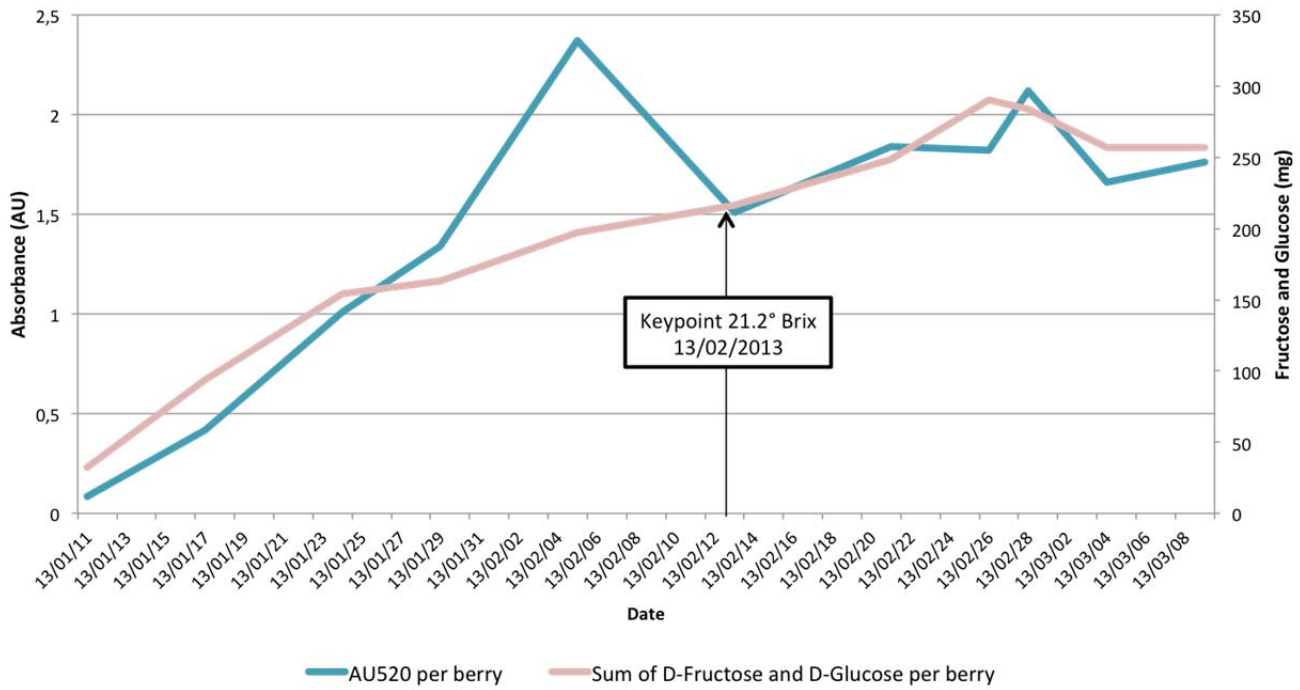

Figure 5.9: Anthocyanin and sugar accumulation

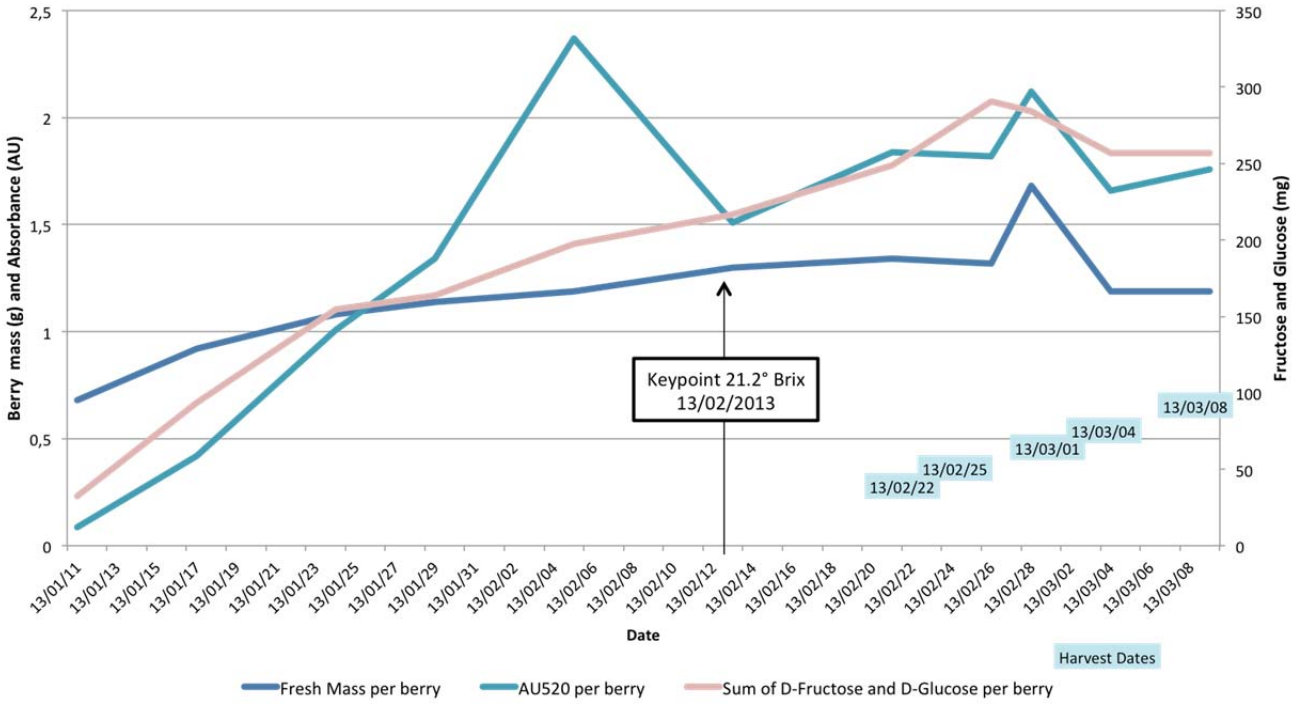

Figure 5.10: Berry fresh mass, anthocyanin and sugar accumulation

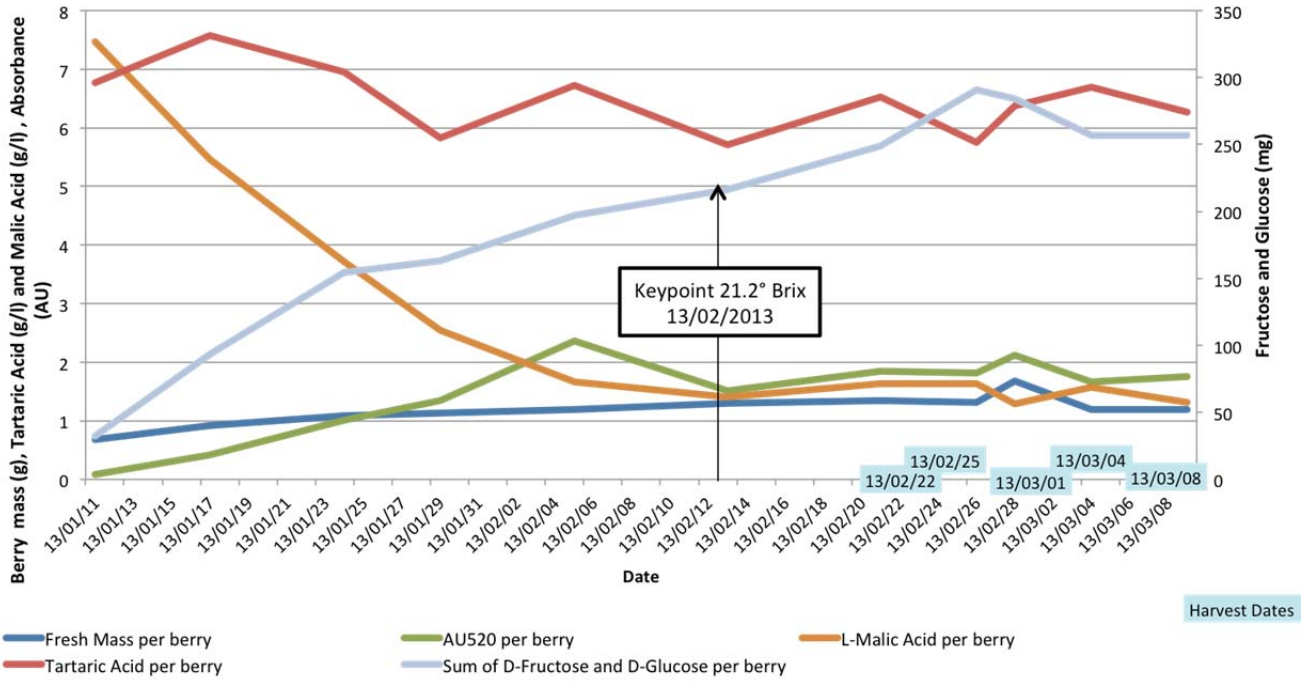

Figure 5.11: Evolution of berry fresh mass, sugars, anthocyanin and organic acid per berry

5.3.2 Must analysis

Brix, pH and TA of the must

The last three harvest dates can be grouped in terms of having the highest mean Brix (Table 8 and Figure B13 in Appendix B.2) and correspond to the highest wine alcohols. Harvests one and two had significantly lower Brix analysis of the must. The results are comparable to the Brix results from berry sampling over the same time period, although the berry samples did not exceed 26

Brix. This could be due to greater extraction or some shriveled berries amongst the harvested berries that lead to an increase in Brix compared to the berry samples.

According to statistically significant differences, two groups can be observed with regard to differences in must pH (Table 8 and Figure B14 in Appendix B.2). Highest pH: Harvests 3, 4 and 5; lowest pH: Harvests 1 and 2. The major factors in pH include total amount of acid that decreased with time, ratio of malic to tartaric acid that decreased to 0.2 and quantity of potassium ions that increases during ripening (Ribéreau-Gayon *et al.,* 2000).

The mean titratable acidity for Harvest 3 was significantly higher than the other harvests (Table 8 and Figure B15 in Appendix B.2).

Table 8: Mean Brix, pH and TA of must

Harvest date	Brix	pH	TA
13/02/22	22.43^{c}	3.40^{b}	5.29^{b}
13/02/25	23.83^{b}	3.38^{b}	5.47^{ab}
13/03/01	25.53^{a}	3.57^{a}	5.62^{a}
13/03/04	26.47^{a}	3.54^{a}	5.26^{b}
13/03/08	25.80^{a}	3.55^{a}	5.27^{b}

5.3.3 Wine analysis results

Classical wine analysis

The wines were analysed (Table 9) in August 2013, six months after harvesting the grapes. The mean wine pH increased with later harvest dates, with the exception of Harvest 5. The pH of each wine was significantly different (see Appendix B.2 Figure B16). There were no significant differences in the titratable acidity of the wines (see Appendix B.2 Figure B17). On comparing the must TA to wine TA the following trends were observed:

- H1 increased 0.3 g/L
- H2 decreased 0.32 g/L
- H3 increased 0.5 g/L
- H4 decreased 0.6 g/L
- H5 increased 0.16 g/L

It is unusual for TA to increase without the addition of tartaric acid and the juice analysis should therefore only be used as an approximate indication (Ribéreau-Gayon *et al.,* 2000).

The alcohol increases significantly in later harvest dates with the exception of the last harvest date, which is not significantly different to Harvests 3 or 4. This trend corresponds to the must Brix content (Table 8, Figure B18 in Appendix B.2).

The volatile acidity in wine made from the last harvest date is significantly higher than the other four harvests' wines (Figure B19, Appendix B.2). This could be due to slower malolactic fermentation in one of the three wines from this harvest date, as well as a small amount of rot in the vineyard that would be more prevalent in the later harvest dates. The highest value is still below the sensory threshold (0.7 g/L – 1.2g/L).

Wine from Harvest 4 has a significantly higher residual sugar than wine from the other harvest dates, followed by Harvests 3 and 5. These last three harvests all had significantly higher starting Brix. The wine with the highest RS (H4) also had the highest Brix, which corresponds to the grape samples with the lowest glucose to fructose ratio (Table 5, Figure B20 in Appendix B.2). The higher RS could be due to more fructose in the must that could not be adequately fermented leaving a higher residual sugar; fructose was, however, not tested in the wines. The findings are similar to those of Bindon *et al.,* (2013) for Cabernet Sauvignon wines where there was a net increase in RS with increase in harvest date.

Table 9: Mean wine pH, TA, alcohol, VA, extract and RS

Harvest date	Wine pH	Wine TA	Alcohol	VA	Extract	RS
13/02/22	3.63^{e}	5.52^{a}	12.87^{d}	0.46^{b}	23.91^{d}	1.35^{c}
13/02/25	3.71^{d}	5.15^{a}	14.07^{c}	0.45^{b}	26.10^{c}	1.40^{c}
13/03/01	3.83^{b}	5.67^{a}	14.90^{b}	0.49^{b}	28.65^{b}	1.65^{b}
13/03/04	3.91^{a}	4.77^{a}	15.94^{a}	0.47^{b}	31.03^{a}	1.98^{a}
13/03/08	3.78^{c}	5.43^{a}	15.48^{ab}	0.57^{a}	29.79^{ab}	1.75^{b}

Tannin and anthocyanin analysis

Table 10: Tannin and anthocyanin results

Harvest date	Tannin (mg/L)	Total red pigment colour (AU)	Wine colour density (AU)	Wine colour hue	Degree of red pigment colouration (%)	Total Phenols (AU)
13/02/22	106.6^{c}	37.83^{c}	13.97^{c}	0.52^{d}	21.08^{b}	58.55^{d}
13/02/25	250.1^{b}	42.33^{b}	16.92^{cb}	0.54^{c}	22.32^{ab}	69.22^{c}
13/03/01	301.6^{ab}	44.19^{b}	18.92^{ab}	0.58^{b}	23.03^{ab}	75.67^{cb}
13/03/04	362.3^{a}	43.88^{b}	21.21^{a}	0.61^{a}	25.53^{a}	77.81^{ab}
13/03/08	254.9^{b}	48.62^{a}	21.19^{a}	0.58^{b}	23.42^{ab}	84.09^{a}

There is a net increase in tannin with later harvest dates (Table 10, Figure B21 in Appendix B.2). Harvests 2, 3 and 5 are not significantly different from each other. It is well known that tannin extractability is influenced by harvest date (Cadot *et al.,* 2012; Bindon *et al.,* 2013). Bindon *et al.,* (2013) hypothesized that the decrease in tannin with later harvest dates could be due to greater concentration of polysaccharides (particularly rhamnogalacturon II) in the must which could lead to aggregate formation and precipitation resulting in a loss of tannin. The tannin increases with harvest date, however, the values of tannin concentration for Cabernet Sauvignon (Bindon *et al.,* 2013) is much higher with a range of 731 to 1088 mg/L compared to Durif (106 to 254 mg/L). The difference in values can be ascribed to different methods of analysis used.

The total phenols were measured at 280 nm, and results ranged from 58-84 AU (Figure B22 in Appendix B.2). This falls into the range (27-104) identified by Somers and Ziemelis (1985) using over 400 red wines. There is a clear increase in total phenolics with harvest date, similarly to the findings of (Bindon *et al.,* 2013).

As expected, wine colour density increases in later harvest dates- corresponding to the literature (Ribéreau-Gayon *et al.,* 2000) although there was no further increase after 4 March 2013 (Harvest 4) (Figure B13 in Appendix B.2)). According to (Ribéreau-Gayon *et al.,* 2000) the range for wine colour intensity is 0.8 to 1.3 AU. In comparison, the range of colour intensity for Durif is high – 1.3 to 2.1. The colour intensity is an indication of the amount of colour and varies with variety and harvest date. In comparison to Bindon *et al.*'s (2013) results, the trend is similar with an increase in wine colour density with an increase in harvest date. However, the highest value at maximum ripeness is 1.45 AU, which is close to the first harvest of Durif.

As expected, wine colour hue increases with later harvests (Figure B24 in Appendix B.2) as there is an increase in polyphenol oxidase activity (Romeyer *et al.,* 1983). Hue is also known to increase with wine ageing (Ribéreau-Gayon *et al.,* 2000). According to literature (Ribéreau-Gayon *et al.,* 2000) the range for wine colour hue is 0.5-0.7 for young wines and can increase up to 1.3 throughout aging. The results of the sequentially harvested wines range from 0.52 to 0.61 and fall into the range. Harvest 4 has the highest hue and also the highest percentage of AU at 420 nm.

The red pigment colour was measured at absorbance at 520 nm with addition of HCl and ranged from 37 to 48 AU (10 mm) (Figure B24 in Appendix B.2). This is far outside the range of Somers and Ziemelis (1985), of 1.3 to 16.2 AU (10 mm) in a study of over 400 Cabernet Sauvignon and Shiraz.

Degree of red pigment at actual wine pH showed significant differences for Harvest 1 (pH 3.63) and Harvest 4 (pH 3.93) (Figure B26 in Appendix B.2). The pH of the wines does not correspond to an increased percentage of red pigment as Harvest 4 pH is significantly higher than that of Harvest 1. The trend shows that the percentage of red pigment increases slightly with harvest date (irrespective of pH). The percentage of red pigment coloration is at a maximum of 25%, which is at the maximum of the range of 5-25% red pigment coloration at wine pH (Cheynier *et al.,* 2006).

Perez-Magarino and Gonzalez-San Jose (2006) observed in young experimental wines that there were a higher percentage of violet tones and colour intensity in wines harvested from a later date, and that these wines were better suited to ageing. However, it was also observed that the latest harvest date was not the highest in colour intensity and percentage of blue. These findings are similar to what is observed in the data; the percentage of AU at 620 nm (Table 11 and Figure 5.12) increased with harvest date and the colour intensity increased with harvest date. However, it did not increase after Harvest 4 (Harvest 4 and Harvest 5 had the same colour intensity) (Gonzalez-Neves *et al.,* 2007; Pérez-Magariño and González-San José 2006).

Table 11: Contribution of each wavelength to colour density

	%Blue	%Yellow	%Red
H1	13.2	29.7	57.1
H2	13.9	30.3	55.8
H3	15.1	31.1	53.8
H4	15.5	32.0	52.6
H5	15.1	31.2	53.7

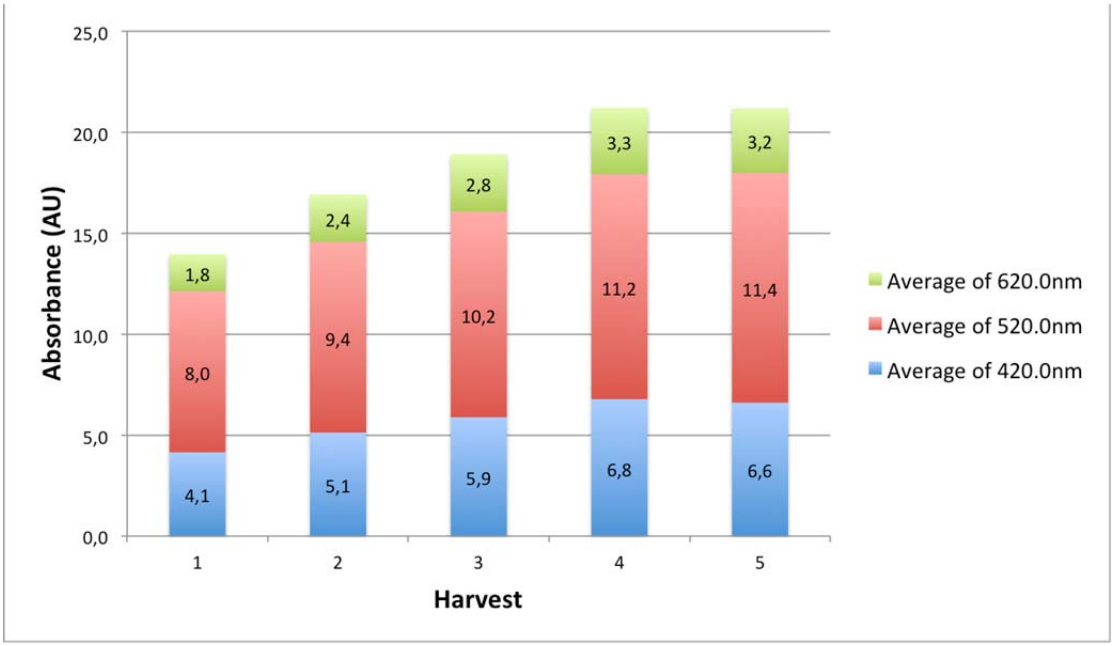

Figure 5.12: Colour density displayed with contribution of each wavelength

There was an increase in wine colour density, hue and red pigment in later harvest dates; however, this does not relate to anthocyanin content of the berries (Table 7). The amount of anthocyanins in the berries is not directly proportional to that in the wine and lower anthocyanin content was observed in later harvest dates. The anthocyanin content of wine depends on the extractability, which can increase as berries soften with ripening (Hunter *et al.,* 2012). The increase in extractability and wine colour intensity with later harvest dates in Cabernet Sauvignon has been shown by Glories (1997). Hunter *et al.,* (2012) found that the ripeness level affects the skin/juice ratio and berry crushing process due to the natural dehydration of berries as they ripen, which in turn affects the extraction process. The anthocyanin profiles were found to be different at different ripeness levels and the ripeness level affected the rate and pattern of extraction (Hunter *et al.,* 2012).

Bindon *et al.,* (2013) observed good relationships between wine colour density and anthocyanins with grape anthocyanins. In this study, similarly to the findings of Bindon *et al.,* (2013), wine tannin showed a poor relationship with grape tannin. Bindon *et al.,* (2013) and Cadot *et al.,* (2012) suggest that ripening influences the extractability of grape and seed tannin, which influences wine tannin.

5.3.4 Sensory analysis

Panel performance

The panel performance for the aromatic descriptors was calculated using the mean R_i which was equal to 0.33 ± 0.10 (Figure 5.14), which is acceptable according to Campo *et al.,* (2010). Based on performance, the number of panelists was reduced from 34 to 31.

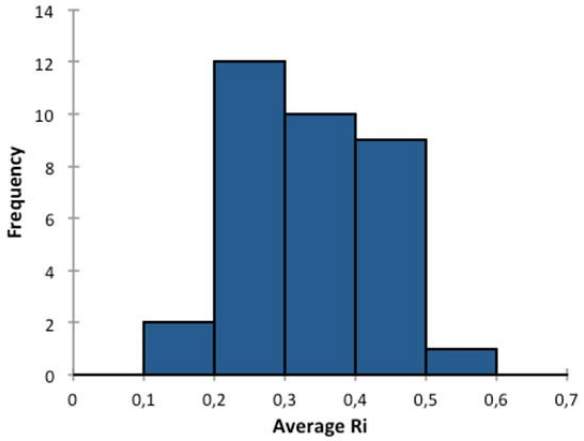

Figure 5.13: Histogram showing average reproducibility index

The panel performance for mouthfeel descriptors was assessed using a three-way ANOVA. The main effects were: wine, panellist and replication. The interaction effects were: panellist by wine, panellist by replication and wine by replication (see results of three-way ANOVA in Table B1 Appendix B.2).

The judge effect and judge x repeat interaction were found significant ($p < 0.0001$). This could be due to judges using the scale differently. Judge x wine interaction showed the judges scored wines similarly and the agreement between judges is good. The wine effect shows the wines were significantly different for astringency, bitterness and alcohol perception. The mean results of sweetness and sourness were not significantly different and have not been included in further PCA analysis. The sensory panel found no significant differences in the perception of sourness between the wines, which correspond to no significant differences in the titratable acidity (Table 9).

Characterization of wine aroma

Wine aromas were grouped into families to help identify trends (Appendix B.1).

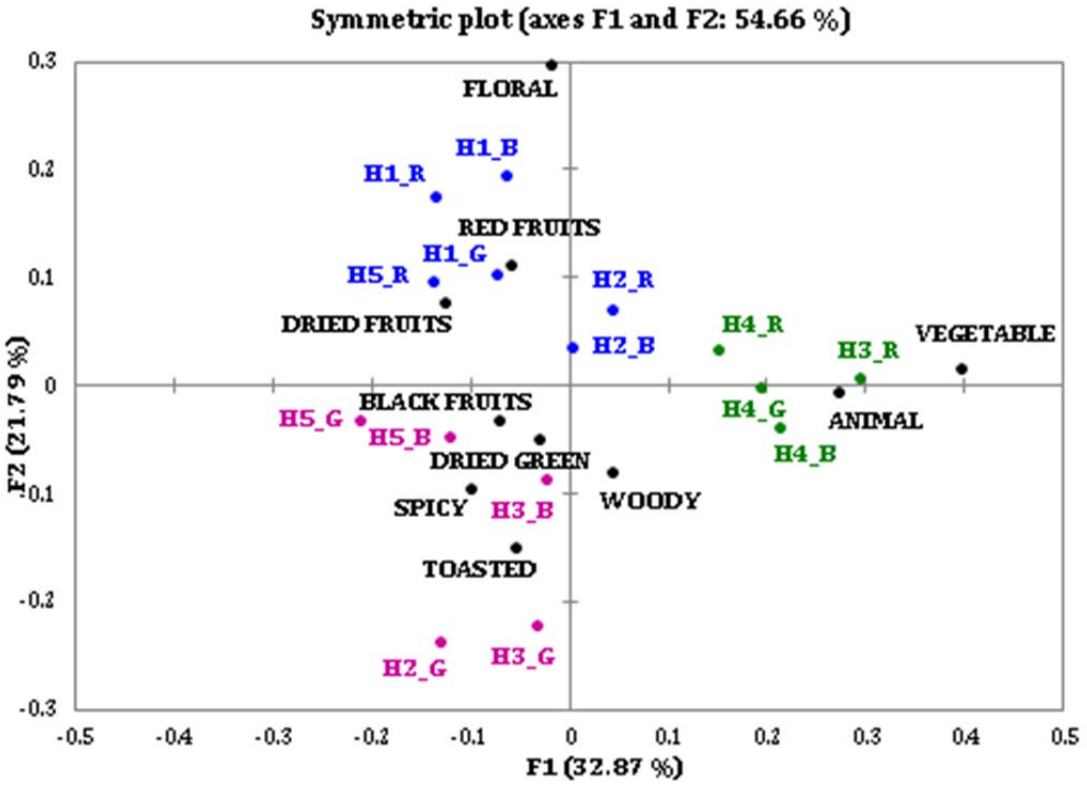

Figure 5.14: Correspondence analysis: Plot of first and second dimension. The first dimension, explaining 32.87% of the variance, opposed black and dried fruit descriptors to vegetable and animal descriptors. The second dimension, explaining 21.79% of the variance opposed toasted aromas to fruity and floral.

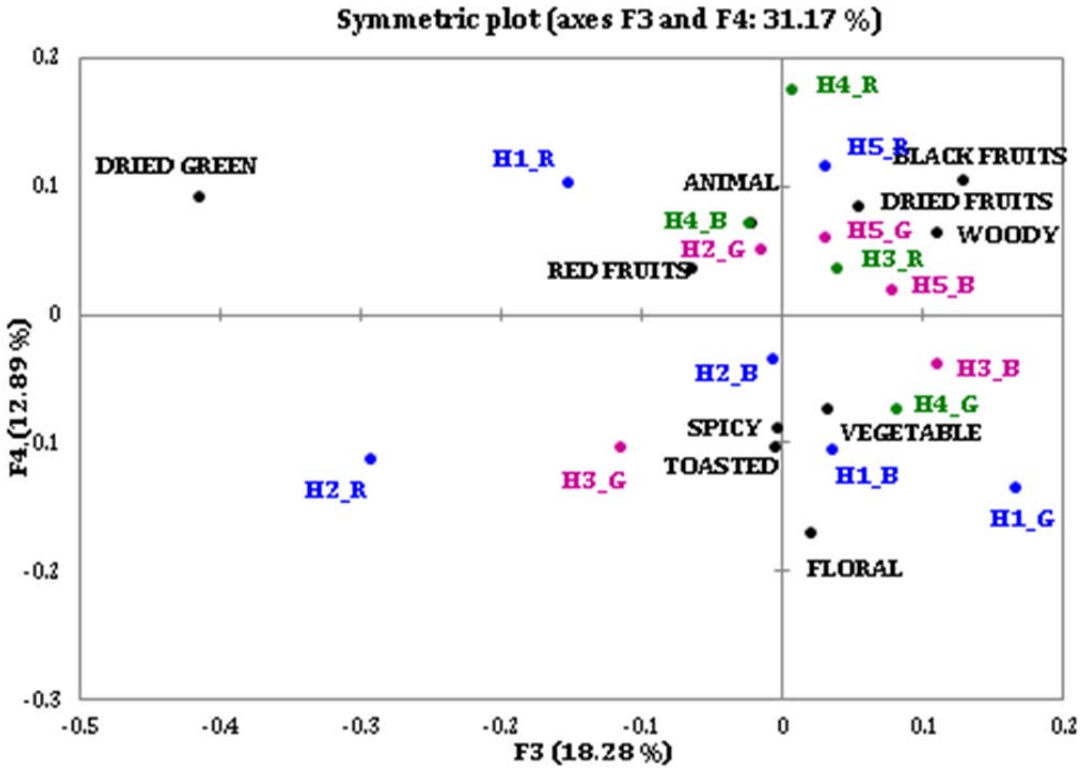

Figure 5.15: Correspondence analyses: Plot of third and fourth dimension. The third and fourth dimension account for 31.17% of the variance

The correspondence analysis showed the projection of wines and descriptors in four dimensions and accounted for 85.8% of the total variance. The first dimension (Figure 5.14), explaining 32.87% of the variance, opposed black and dried fruit descriptors to vegetable and animal descriptors. The second dimension (Figure 5.14), explaining 21.79% of the variance opposed toasted aromas to fruity and floral. The third and fourth dimensions account for 31.17% of the variance (
Figure 5.15). The hierarchical cluster analysis produced three main groups separated by the axis of F1 and F2 and represents only 54.6% of the variation (Figure 5.16).

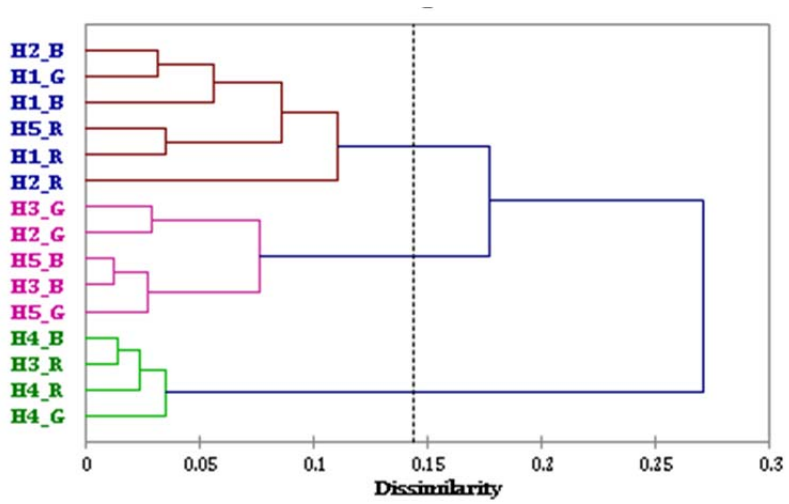

Figure 5.16: Hierarchical cluster analysis: dendrogram of wines

The colours indicate the groups that are least dissimilar in the hierarchical cluster analysis. Harvest 1 (H1_B, H1_R, H1_G) as well as Harvest 2 (H2_R, H2_B) points are within close proximity to red fruits showing an association with red fruits (Figure 5.14). Harvests 1 also show an association with floral descriptors. H2_G is an exception and shows an association with toasted descriptors (Figure 5.14) and animal descriptors (Figure 5.16). These 5 wines (H1_B, H1_R, H1_G, H2_R, H2_B) are grouped together (Figure 5.16) with one wine from Harvest 5 (H5_R) which was closely associated with dried and red fruits.

Two biological repeats of Harvest 3 (H3_B, H3_G) are associated with dried green, spicy and toasted aromas and one repeat is associated with vegetable and animal aromas (H3_R). Harvest 4 (H4_R, H4_G, H4_B) is associated with animal and vegetable aromas (Figure 5.14). These three wines (H4_R, H4_G, H4_B) are grouped with one wine from Harvest 3 (H3_R) and were characterized by vegetable and animal attributes. Two biological repeats of Harvest 5 are closely associated with black fruits (H5_G and H5_B) and one with dried fruits and red fruits (H5_R) (Figure 5.14). These two wines from Harvest 5 (H5_G and H5_B) are grouped with two wines from Harvest 3 (H3_G, H3_B) and H2_G. This group showed an association with black fruit, dried fruit and dried green and toasted notes (Figure 5.14) and woody attributes (Figure 5.16). Within this group H3_G and H2_G are least dissimilar (Figure 5.16) and could be considered as a subgroup. Differences between replicates, even fermentation replicates, is not unusual. Bindon *et al.,* (2014) found replicate effects for various attributes such as fruit, dark fruit, sewage and fresh green flavours.

The difference in citation frequency of the different aroma attributes was small and there were no significant differences between citation frequencies of the attributes. Figure 5.17 highlights the differences between the means of the biological repeats and some trends can be observed which confirm the findings of the correspondence analysis (see Appendix B.2 Figure B27, B28, B29, B30, B31 for more detail).

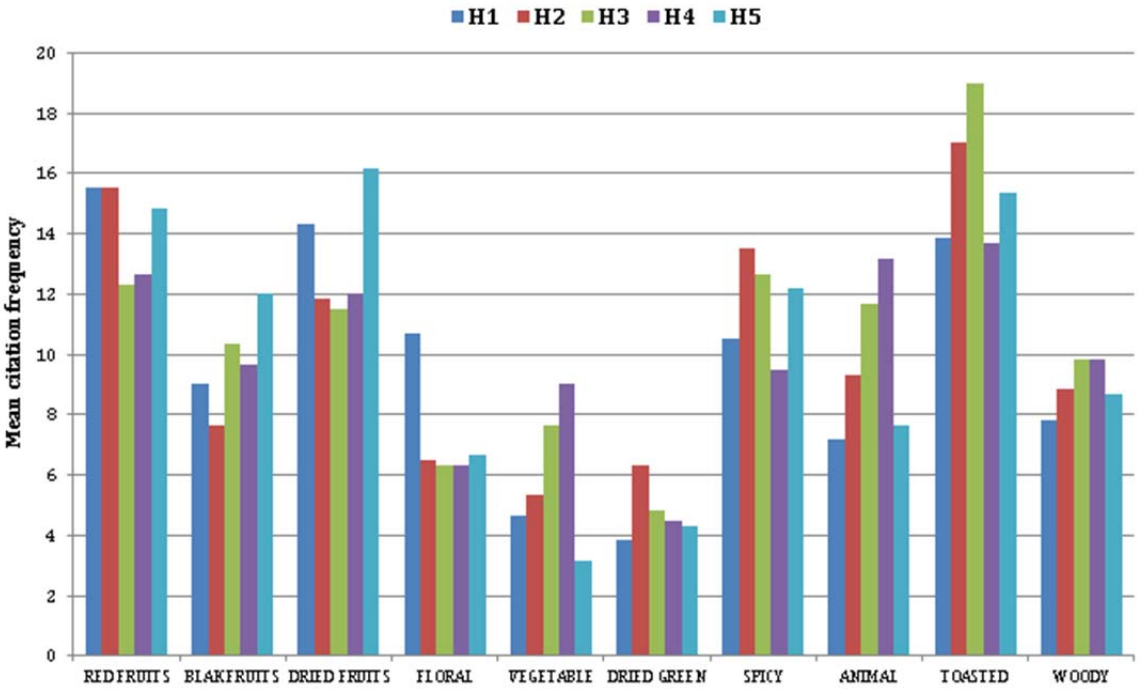

Figure 5.17: Mean citation frequencies for each harvest date per attribute

Harvests 1 and 2 had the highest citation frequency for red fruits. Harvest 5 had the highest citation frequency for black fruits and dried fruits. This is in agreement with the findings of Bindon *et al.,* (2014) and Antalick *et al.,* (2015) where earlier harvest dates were more highly rated for red fruit and fresh green, and wines from later harvest dates were higher rated for dark fruits and overall fruit. Dried fruits highest citation frequencies were in Harvests 1 and 5. Dried fruit was one of the highest cited attributes for H5, particularly for H5_R. The highest citation frequency for floral notes was Harvest 1. Although not tested in this study, Bindon *et al.,* (2014) found beta ionone (violet aroma) present only in the earliest harvest (for Cabernet Sauvignon). Analysis of the norisoprenoids in the sequentially harvested wines could have been an interesting addition to the sensory results. Dried green and spicy aromas had the highest citation frequency in H2, decreasing thereafter. Toasted and woody attributes increased with harvest date peaking at H3 and decreasing thereafter. In general the citation of toasted notes was high, this included vanilla as part of the grouping, which had the highest citation frequency of all attributes. Vegetable attributes were highest rated in H4 and animal attributes showed a similar trend increasing to a peak in H4 and then decreasing dramatically in H5. Vegetable aromas (the group used for assessment consisted of canned artichoke, asparagus, bean, olive and fresh celery, green pepper and cooked cabbage) can be from the presence of dimethyl sulphide. Bindon *et al.,* (2014) found wines from later harvest dates had higher concentrations of dimethyl sulphide and, together with

increased ester formation these were the two main driver compounds for the dark fruit aroma in wines from later harvest dates.

The decrease in fruity aromas in Harvests 2, 3 and 4 could be due to interactive effects of the wine matrix as these wines have high citations of toasted, spicy, animal, woody and vegetable attributes. Fruit is hidden by other attributes and can be masked by vegetative odours (Bindon *et al.,* 2013; Escudero *et al.,* 2007). Fruity aromas can also be affected by astringency. Cliff *et al.,* (2012) found increasing astringency with grape seed extract which led to a decrease of fruity aroma and an increase in woody and earthy aromas. The observations in the data are in agreement — Harvest 4 had the highest rating for astringency and also the highest frequency of vegetable, woody and animal aroma. Forest floor (in the same family as earthy) was rated higher and fruity attributes rated lower in the more astringent wines compared to the other wines. Goldner *et al.,* (2011) found the intensity of fruity and floral aromas decreased with an increase in polyphenols, and in these sequentially harvested wines there is an increase in total phenolics with increasing harvest date (see Accumulation of total anthocyanins and total phenolics). This, however, does not explain the high citation of red, black and dried fruit in Harvest 5 which has higher phenolics. Phenolics could, however, add to the suppression of fruity aromas in H3 and H4.

On comparing sequentially harvested wines, the impact of increasing Brix on yeast metabolism and the effect of grape composition on yeast metabolism needs to be taken into consideration. The wine volatile composition of sequentially harvested wines and subsequent sensory analysis of wines has been explored (Bindon *et al.,* 2013; Boss *et al.,* 2014; Šuklje *et al.,* 2014) and remains a complicated subject. Šuklje et al., (2014) used Common Components Analysis to determine the main driver for the differences in wine composition and found harvest date was one of the most important factors. This type of statistical analysis is important in further studies to understand the complexity of the main drivers of wine composition.

Characterization of wine taste and mouthfeel

There were no significant differences between harvest dates in wine sweetness or sourness (Appendix B.2 Table B1). A correspondence analysis (Figure 5.18) and a dendrogram (Figure 5.19) were done for bitterness, alcohol and astringency perception. The grouping of the wines based on dissimilarity (refer to dendrogram, Figure 5.19) is a follows:

- Group 1 all three wines from Harvest 1.
- Group 2 two wines from Harvest 2 and all the wines from Harvest 3, as well as H5_G.
- Group 3 wines from Harvests 4 and 5, as well as H2_G.

This grouping shows that there is a difference in perceived mouthfeel between harvest dates. Harvest 1 is the most different from the other harvest dates. Harvests 2 and 3 are similar to each other and Harvests 4 and 5 are similar to each other. The earlier harvest dates are grouped and the later harvest dates are grouped.

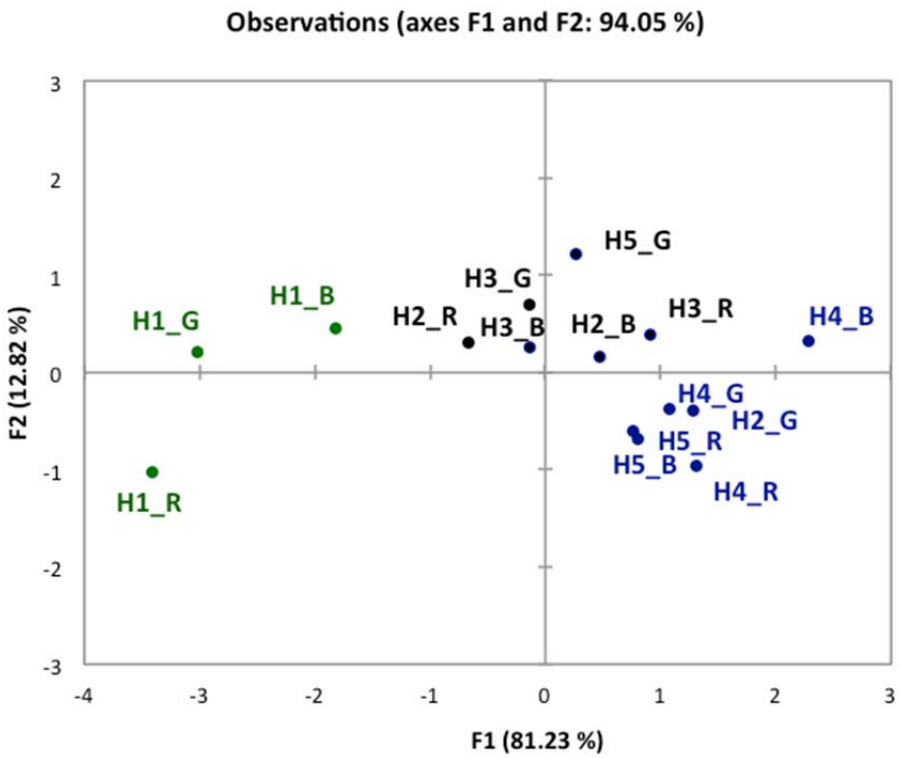

Figure 5.18: Correspondence analysis of wine mouthfeel (bitterness, alcohol and astringency perception) explaining 94.05% of the variance. The first dimension (explaining 81.23% of the variance) opposes early harvest dates (H1) and later harvest dates (H4 and H5)

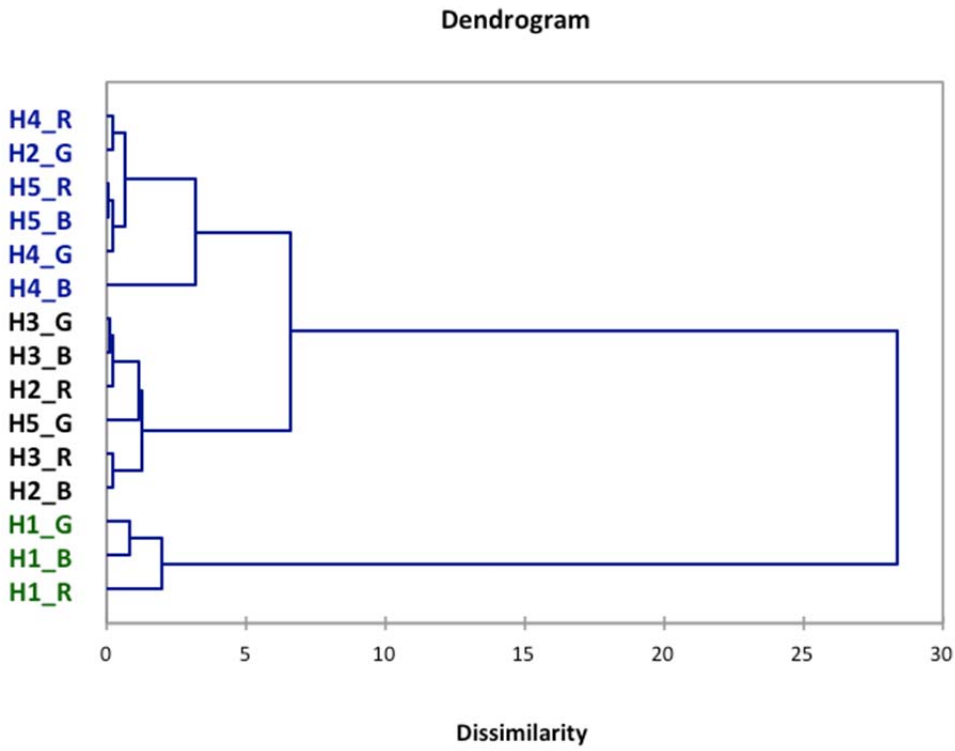

Figure 5.19: Hierarchical cluster analysis: dendrogram of the wines. Group 1 all three wines from Harvest 1, Group 2 two wines from Harvest 2 and all the wines from Harvest 3, as well as H5_G. Group 3 wines from Harvests 4 and 5, as well as H2_G.

The sensory panel found no significant differences in the perception of sourness and sweetness of the wines (Figure 5.20). This corresponds to the analysis of the wine titratable acidity, showing no significant difference between the wines acidity (Table 9). The analysis of the wines RS showed there was a significant difference in RS (Table 9). H4 had the highest RS followed by H5 and H3, then H2 and H1. The range of RS was small from 1.25 to 1.9 g/L. The wines were statistically significantly different in RS analysis. However, it would be unrealistic to expect the panel to pick up a difference in 0.8 g/L range, taking into account the effect astringency has in these wines.

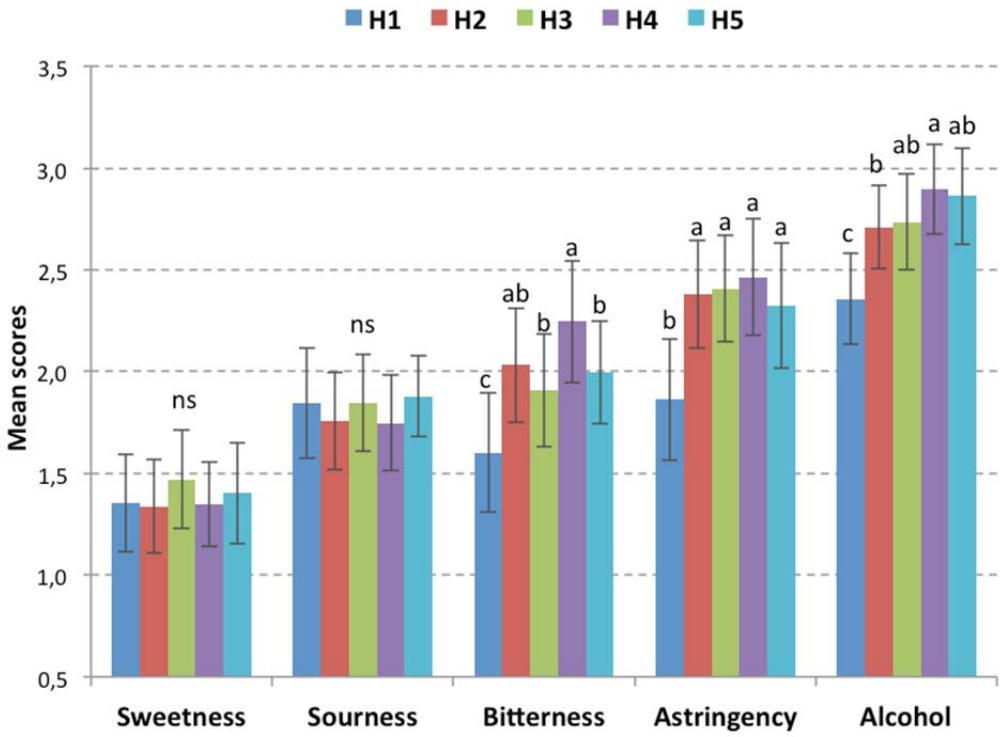

Figure 5.20: Mean scores for mouthfeel for each harvest date

Although the mean score of H4 was not significantly different to H3 and H5, the sensory panel found the perception of alcohol highest in wine H4 which corresponds to the wine with the highest alcohol analysis (15.94 %). The panel found H1 significantly lower in perception of alcohol than the other dates which correspond to the wine analysis (Table 9).

Bitterness, astringency and alcohol perception increased with harvest date. H1 was significantly less bitter, less astringent and had a lower perception of alcohol than the other harvest dates which correlates to the analysis of the wines: lowest tannin 106.6 mg/L, lowest total phenolics 58.55 AU and lowest alcohol 12.8% v/v. There is an increase in perceived astringency (although not significant) from Harvest 2 to 4 and then a slight decrease for Harvest 5. This corresponds to the trend of tannin concentration in the wines (Table 10). Harvest 4 had the highest perceived alcohol, bitterness and astringency ratings (although not significantly different), which correlates to highest alcohol analysis 15.9% v/v and the highest tannin analysis 362 mg/L.

Alcohol, pH, TA, viscosity and tannin concentration can affect the perception of astringency in wine. Wine pH can have a larger influence on the perception of astringency than increasing wine

TA (Sowalsky and Noble, 1998), however the lower pH of H1 and H2 did not correlate to a higher perceived astringency wine. Wine TA can be excluded in understanding the factors that affect perceived astringency, as there was no significant difference between wines. Tannin concentration was the main driver for perceived astringency in these wines, which was amplified by increasing alcohol concentration. Alcohol has been linked to perception of astringency (Obreque-Slíer *et al.,* 2010) and in this case the wine with the lowest alcohol also has the lowest perceived astringency (H1). Literature has shown that fruity aroma is inversely related to astringency and bitterness (Sáenz-Navajas *et al.,* 2010), and in this case the wine with the highest citations of red fruit had the lowest perceived astringency.

The most dissimilar wines were the group of H1 when compared to the group of H4 and H5 (Figure 5.19). The perception of astringency appears to be similar for H2, H3, H4 and H5. These similarities could be due to the difficulty in perceiving differences in wines that are quite similar, particularly such astringent wines, or there are truly no differences. It is possible that with such similar wines it would be more beneficial to look at astringent sub-qualities for the description, as the type of feeling of the tannin might be more important than the average intensity rating, for example, using descriptors of the mouthfeel wheel to describe the astringency (drying, chalky, grainy, furry, aggressive, hard, etc. (Ma *et al.,* 2014; Gawel *et al.,* 2000)).

What consistent aromatic wine descriptors can be used to describe wines from sequential harvest as fresh and mature fruit?

The literature has shown the differences in aroma profiles of sequentially harvested wines with earlier harvests attributed to red fruit (strawberries, red cherry, raspberry, redcurrant), fresh green (green stalks, leafy, fresh grass, tomato leaf), herbaceous, and unripe plum. Later harvests are associated with dark fruit (blackberry, black cherry), overall fruit (plum, cherry, blackberry, strawberry, raspberry, blackcurrant), and stewed fruit. Overripe is characterized by jammy, raisin and dried fruit notes (Šuklje *et al.,* 2014; Bindon *et al.,* 2013; Deloire, 2013,; Deloire, 2011).

The aim of the project was to determine the best picking date in order to have the wine that most represents a Fresh Fruit style and Mature fruit style wine considering the descriptors mentioned above. The most representative fresh fruit style wine was H1 with the highest red fruit and floral aromas and was also described by black fruit and dried fruit. The most representative mature fruit style wine was H5 with the highest black fruit and dried fruit aromas, the wine was also described by red fruit and toasted aromas.

The berry aromatic sequence for Durif wines for fresh fruit harvesting 10 days after the keypoint is reached and 23 days after the keypoint for mature fruit. To wait for harvesting for more than 23 days would not be beneficial, as the wine will have even more notes associated with overripe character. From experience, Durif seems to turn overripe quickly and the high citation frequency of dried fruit character at the mature fruit stage could indicate the window period for harvesting is small.

Wine sensory attributes for Durif wine styles

The most frequently cited terms used to describe Durif wines are listed below (Table 12). This includes sensory analysis results from all the different harvest dates from both 2012 and 2013 vintages. The aromas in bold are the descriptors which were highly rated. Interestingly, vanilla was the most cited descriptor in both 2012 and 2013 followed by toasted. It is possible to conclude that the descriptors best used to describe Durif wines are alcohol, black pepper, blackberry, blackcurrant, caramel, cherry, dried apricot, honey, leather, prune, raspberry, toasted, vanilla and woody. It should be taken into account that the wines were made in the experimental cellar (unwooded) and further study of commercial Durif from other wine growing regions in South Africa is needed to understand the potential aromatic profile of the wines.

Table 12: List of most cited descriptors for Durif wines. Wines in bold were the most cited descriptors in both 2012 and 2013 vintages.

Family	Descriptor cited
Red fruits	Redcurrant
	Strawberry
	Raspberry
	Cherry
Black fruits	**Blackcurrant**
	Blackberry
Dried fruits	**Dried apricot**
	Dried fig
	Prune
Floral	**Honey**
Hay/Dried Grass	Hay/Dried Grass
Animal	**Leather**
Spice	Bay leaf
	Black pepper
	Clove
Forest Floor	Humus/Earthy
Toasted	**Toasted**
	Caramel
	Vanilla
	Woody/planky
Tobacco	Tobacco
Other	Sulphur
	Alcohol

5.4 Conclusion

The potential wine profiles that can be achieved from Durif grapes were evaluated and harvest timing to identify fresh and mature fruit style wines was identified. The main aromatic attributes associated with Durif wine in general, and with fresh and mature fruit profile in particular, were identified. The mouthfeel of Durif wines was investigated.

The data shows that here are significant changes (refer to Appendix for p-value) in the berry composition during ripening and this is translated into differences in the wine matrix between harvest dates, seen both in chemical composition and from the sensory evaluation. However, the grape composition is not directly related to the wine composition, which is widely written about - the impact of extraction during fermentation (with increasing levels of ethanol) on tannin and anthocyanin extractability is a factor and the impact of grape composition (which changes with later harvest dates) on yeast metabolism plays a role in wine composition. In literature, it is clear the wine volatile matrix also changes with harvest date (Cadot *et al.,* 2012; Bindon *et al.,* 2013; Šuklje *et al.,* 2014) and the relationships with harvest timing are complicated. The sensory analysis confirmed the impact of harvest date on wine aroma and mouthfeel; for example, the wine analysis corresponded to sensory analysis for TA and sourness, tannins and bitterness, astringency and alcohol. This study is the first to uncover the attributes associated with Durif wines in South Africa using sequential harvest.

The study using sequential harvest and ripening stages has allowed trends to be observed for fresh fruit and mature fruit style wines. The volatile composition was not studied. However, from the trends observed for the descriptors of the wines it seems there is a berry aromatic evolution for Durif. Harvest timing could be defined, as picking 10 days after the keypoint produced fresh fruit style wines and 23 days after the keypoint produced mature fruit style wines. The study should be expanded to different sites and over several vintages to confirm the research.

The effect of harvest timing on mouthfeel was explored and there is an increase in perceived astringency and alcohol with an increase in harvest date. There were trends (not significantly different) in aroma attributes. Harvest 1 had the highest citations for red fruit and floral notes. Harvest 2 had the highest citations for red fruit, dried green and spicy aromas with toasted notes and the lowest citations for black fruit. The wines of Harvest 3 had the highest frequency of citation of all the wines for toasted and woody notes. Spicy, vegetable and animal aromas also describe this wine. The wines from Harvest 4 showed the highest frequency of vegetable, woody and animal aroma citations compared to other harvest dates. The wine from Harvest 5 had the highest citation frequency of black and dried fruits compared to other wines. Harvest 5 was also associated with aromas of red fruit, toasted notes and spice.

The analysis of Durif wine composition highlighted that tannin concentration is not as high as other cultivars, although the wine is perceived as very astringent. The anthocyanin concentration in wine and the total red pigment colour are high. The influence of anthocyanins on astringency,

other than in the incorporation into tannin structures which may lead to a decrease in astringency (Cheynier *et al.,* 2006), requires further study in wines and model wines (Vidal *et al.,* 2004).

The increase in wine colour density with harvest date can be further explored practically in cellars. Durif has high colour intensity. The wines at later harvest date have a greater percentage of AU at 620 nm (blue). Pérez-Magariño and González-San José (2006) observed that wines with higher percentage blue and higher colour density as young wines had better potential for ageing. Grapes can be harvested at different ripeness levels for different purposes, using mature fruit style wines to barrel age and fresh fruit wines for early bottling.

5.5 Bibliography

Antalick, G., Šuklje, K., Blackman, J.W., Meeks, C., Deloire, A. and Schmidtke, L.M., 2015. Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry,* 63(18) pp. 4664-72.

Bindon, K., Varela, C., Kennedy, J., Holt, H. and Herderich, M., 2013. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry,* 138(2–3), pp. 1696-1705.

Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M. and Francis, I.L., 2014. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry,* 154(0), pp. 90-101.

Boss, P.K., Böttcher, C. and Davies, C., 2014. Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *American Journal of Enology and Viticulture,* 65(3), pp. 341-353.

Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V., 2012. Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. *Analytica Chimica Acta,* 732, pp. 91-99.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference,* 21(1), pp. 44-55.

Campo, E., DO, B.V., Ferreira, V. and Valentin, D., 2008. Aroma properties of young Spanish monovarietal white wines: a study using sorting task, list of terms and frequency of citation. *Australian Journal of Grape and Wine Research,* 14(2), pp. 104-115.

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J., SARNI-MANCHADO, P. and FULCRAND, H., 2006. Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture,* 57(3), pp. 298-305.

Cliff, M.A., Stanich, K., Edwards, J.E. and Saucier, C.T., 2012. Adding grape seed extract to wine affects astringency and other sensory attributes. *Journal of Food Quality,* 35(4), pp. 263-271.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. and Gerós, H., 2007. *Biochemical changes throughout grape berry development and fruit and wine quality.*

Coombe, B.G., 1992. Research on development and ripening of the grape berry. *American journal of enology and viticulture.,* 43(1), pp. 101.

Deloire, A., 2013. *Wine aroma and grapevine berry ripening: How to capture the complexity. Winetitles, Wine & Viticulture Journal 28(5).*

Deloire, A., 2011. The concept of berry sugar loading. *Wynboer,* 257, pp. 93-95.

Escudero A, Campo E, Fariña L, Cacho J,Ferreira V, 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry,* 55(11), pp. 4501-10.

Gawel, R., Oberholster, A. and Francis, I.L., 2000. A mouth-feel wheel: terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research,* 6(3), pp. 203-207.

Glories, Y., 1984. The colour of red wines. Part 2. Measurement, origin and interpretation. *Connaissance de la Vigne et du Vin (France),* 18(4). pp.195-217.

Goldner, M., Di Leo Lira, P., Van Baren, C. and Bandoni, A., 2011. Influence of polyphenol levels on the perception of aroma in Vitis vinifera cv. Malbec wine. *S.Afr.J.Enol.Vitic,* **32**(1),.

Gonzalez-Neves, G., Franco, J., Barreiro, L., Gil, G., Moutounet, M. and Carbonneau, A., 2007. Varietal differentiation of Tannat, Cabernet-Sauvignon and Merlot grapes and wines according to their anthocyanic composition. *European Food Research and Technology,* 225(1), pp. 111-117.

Guidoni, S. and Hunter, J.J., 2012. Anthocyanin profile in berry skins and fermenting must/wine, as affected by grape ripeness level of Vitis vinifera cv. Shiraz/R99. *European Food Research and Technology,* 235(3), pp. 397-408.

Hagerman, A.E. and Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry,* 26(4), pp. 809-812.

Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y. and Li, H., 2014. A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science & Technology,* 40(1), pp. 6-19.

Obreque-Slíer, E., Peña-Neira, A. and López-Solís, R., 2010. Enhancement of both salivary protein-enological tannin interactions and astringency perception by ethanol. *Journal of Agricultural and Food Chemistry,* 58(6), pp. 3729-3735.

Pérez-Magariño, S. and González-San José, M.L., 2006. Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chemistry,* 96(2), pp. 197-208.

Pirie, A. and Mullins, M., 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. *American Journal of Enology and Viticulture,* 28(4), pp. 204-209.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D., 2000. *Handbook of enology. The chemistry of wine and stabilisation and treatments*, Vol. 2.

Romeyer, F.M., Macheix, J.J., Goiffon, J.J., Reminiac, C.C. and Sapis, J.C., 1983. Browning capacity of grapes. 3. Changes and importance of hydroxycinnamic acid-tartaric acid esters during development and maturation of the fruit. *Journal of Agricultural and Food Chemistry,* 31(2), pp. 346-349.

Ryan, J. and Revilla, E., 2003. Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry,* 51(11), pp. 3372-3378.

Sáenz-Navajas, M., Campo, E., Fernández-Zurbano, P., Valentin, D. and FERREIRA, V., 2010. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry,* 121(4), pp. 1139-1149.

Singleton, V., 1966. The total phenolic content of grape berries during the maturation of several varieties. *American Journal of Enology and Viticulture,* 17(2), pp. 126-134.

Somers, C. T. and Evans, M.E., 1977. Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO2,“chemical age”. *Journal of the science of food and agriculture,* 28(3), pp. 279-287.

South African Wine Laboratories Association (SAWLA), 2002. Methods for analysis for wine laboratories. *South African Society for Enology and Viticulture*.

Sowalsky, R.A. and Noble, A.C., 1998. Comparison of the effects of concentration, pH and anion species on astringecy and sourness of organic acids. *Chemical senses,* 23, pp. 343-349.

Šuklje, K., Antalick, G., Meeks, C., Blackman, J.W., Deloire, A. and Schmidtke, L.M., 2014. Optimising harvest date through use of an integrated compositional and sensory model: a proposed approach for better understanding of ripening of autochthonous varieties. *2nd International Oneoviti symposium,* November 2014.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V. and Waters, E., 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta,* 513(1), pp. 57-65.

Chapter 6

General discussion and perspectives

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6.1 General discussion

The goal of this research project was to assess Durif wine profiles using grapes from Fairview Winery (Paarl, South Africa). Grapes were harvested at different stages of ripeness on different dates (sequential harvesting). The choice of harvest dates influences grape composition which, in turn, influences wine composition. In this research project, sequential harvesting was used to determine Durif's potential ripening aromatic sequence (Deloire, 2011). The grape ripening sequence or timing could lead to different wine styles, which can be achieved by determining harvest time/windows using sugar loading as a berry physiological indicator. These wine styles include a fresh fruit (FF) style, a pre-ripe style (what could sometimes be called neutral due to a lack of fruitiness in the wine or wine with a dominance of spicy aromatic notes), a mature fruit (MF) style and later an over-ripe style (OR). The wines were sensorially assessed using the citation frequency method (Campo *et al.,* 2010), a new method at the University of Stellenbosch (Stellenbosch, South Africa).

The sensory analysis was used to determine which of the sequentially harvested wine attributes correspond to fresh fruit and mature fruit style wines, and whether there was a ripening flavour evolution. The aim was to determine the main aromatic attributes associated with Durif wines in South Africa for each harvest time. The most highly cited attributes for each sequentially harvested wine were identified, providing an overview for Durif wines in general. Durif is well known for its astringent and robust tannins. Another aim of the project was to determine whether the mouthfeel and perception of astringency changes with harvest timing. Descriptive analysis was used to rate the intensity of astringency, bitterness, sweetness, sourness and alcohol perception of the wines.

The goal was to calibrate the model of berry sugar accumulation and to provide practical solutions to determine picking windows/dates in order to assess wine style diversity from a single vineyard, using sequential harvesting, which can then be applied commercially to ensure consistency in wine styles across different vintages.

The research project was carried out over two vintages (2012 and 2013). The first vintage (2012) was used as a diagnostic year in order to understand the vineyard and the potential wine styles. The differences illustrated in 2012 between the high vigour vines and low vigour vines highlight the importance of having a homogenous plot for this research project and for commercial use. The effect of vine vigour on gas exchange and resource utilization, as well as the different water use efficiencies of different vigours, lead to different yields and grape composition (Zerihun *et al.,* 2010).

The second vintage (2013) was used to focus and elaborate on the results from 2012 using a different plot layout where all the vines had similar vigour and only leaf removal as canopy management was applied in the fruit zone (leaf removal and lateral shoot removal were used in 2012).

Berry composition was analysed (fructose, glucose, tartaric acid, malic acid, total anthocyanins and total phenolics) and it was shown that there were significant changes in berry composition during ripening. The evolution of berry composition during the ripening period highlights the importance of optimization of the picking date. The wine composition, however, is not directly related to grape composition as it is compounded by the impact of extraction during fermentation (with increasing levels of ethanol with increasing sugar levels when using sequential harvesting) on tannin and anthocyanin extractability and yeast metabolism (Gil *et al.,* 2015; Fournand *et al.,* 2006). Must composition (pH, TA, Brix) was monitored and showed significant differences between early and later harvest dates.

Sugar accumulation was measured in both vintages. A strong correlation (R = 0.91) was found between the sum of D-fructose and D-glucose (mg/berry) and the calculated sugar content (mg/berry) (2013 results). This confirms the equation used to estimate the sugar per berry is accurate, even though it is considered an estimation depending on seed volume and distribution of sugar in the pulp (Deloire 2011; 2012; 2013) and can be used during harvest time to make harvest decisions when time-consuming laboratory analysis to determine glucose and fructose content is not convenient.

A plateau of sugar per berry accumulation was reached in 2012 (in the low vigour section of the vineyard) and in 2013. In 2013 the keypoint was reached on 13 February 2013 (1650 GDD from 1 September 2012, Eicchorn Lorenz scale 5-7) at 216 mg glucose and fructose per berry. In 2012 the low vigour section reached keypoint on 6 February 2012 (1497 GDD from 1 September 2011, Eicchorn Lorenz scale 5-7) at 210 mg glucose and fructose per berry.

In 2012, the wine composition was analysed and a significant increase in tannin, total red pigment colour and wine colour density with later harvest dates was found. Wine colour hue increased from H1 to H4, but was not statistically significant and the degree of red pigment colouration showed no statistically significant differences. In 2012, the differences in wine composition between H1 and H4 were evident. The differences between H2 and H3 were less obvious due to the way the harvests were grouped for statistical analysis (for example, H2 included the second harvest from different vigours and these different vigours were harvested on different dates). This made comparisons between the 2012 and 2013 vintages difficult, but trends between early and late harvested wines in 2012 could be observed.

The wine composition in 2013 wines was studied and significant differences were found for wine pH, alcohol, VA and RS for wines from different harvest dates. No significant differences were found in wine TA between harvest dates. In the 2013 vintage there was a significant increase in total phenolics, wine colour hue, wine colour density, total red pigment colour, degree of red pigment colouration and tannin from the earliest to the later harvested wines in agreement with the literature (Ribéreau-Gayon *et al.,* 2000; Bindon *et al.,* 2013; Cadot *et al.,* 2012). However, wines from the latest harvest did not necessarily have the highest values for tannin, wine colour density, wine colour hue and degree of red pigment colouration. Durif has a very high concentration of red pigment colour and high colour intensity, increasing with later harvests. The wines at later harvest dates have a greater percentage of blue as measured by absorbance at 620 nm. Pérez-Magariño *et al.,* (2006) observed that wines with higher percentage of blue and higher colour density as young wines have better potential for ageing. Wines can be harvested at different ripening stages/levels for different purposes, using mature fruit style wines to barrel age and fresh fruit wines for early bottling.

The wine volatile matrix also changes with harvest timing (Cadot, *et al.,* 2012; Bindon *et al.,* 2013; Šuklje *et al.,* 2014). The sensory analysis highlighted significant differences in the mouthfeel of the wines in 2012 and 2013. The study showed that harvest timing has an effect on the mouthfeel of wines. There was an increase in perceived astringency and alcohol with later harvest dates in agreement with Bindon *et al.*'s (2013) findings. This corresponded to higher tannin and alcohol contents in the most astringent, bitter and alcoholic wines. There were no differences in sourness and sweetness perception in the sensory analysis of 2013 wines, which corresponded to no significant differences in the wine TA and very small difference in the RS of the wines. In the sensory analysis for wine from the 2012 vintage, the judges were not consistent in the ratings for sweetness and therefore it was excluded.

The aroma profile did not show statistically significant differences between the citation frequencies of the attributes in 2012 or 2013. The aroma profile could be assessed in terms of the correspondence analysis (2013 results) accounting for 85.8% of the variance and trends of the citation frequencies for each harvest date could be observed for each vintage. The frequency of citation method is not suited to highlighting significant differences between very similar wines (Nell, 2015; Campo *et al.,* 2010). It was, however, beneficial to have an extensive attribute list (unlike descriptive analysis which reduces the attribute list) in order to determine the most highly cited attributes for Durif wines in general, as well as determining the wines most associated with the fresh fruit and mature fruit style attributes. It is possible to conclude the descriptors best used to describe the aromatic profile of Durif wines in general are alcohol, black pepper, blackberry, blackcurrant, caramel, cherry, dried apricot, honey, leather, prune, raspberry, toasted, vanilla and woody. These were the most highly cited attributes in 2012 and 2013. It should be taken into account that the wines were made in the experimental cellar (unwooded) and further study of commercial Durif from other wine growing regions in South Africa is needed to understand the full potential of the aromatic profile of Durif wines.

From the trends observed in the frequency of citation method it is suggested that the berry aromatic ripening sequence for Durif exists and fresh or mature fruit style wines can be made. Harvest timing could be defined as picking ten days after the keypoint produces fresh fruit style wines and twenty-three days produces mature fruit style wines according to the results of the 2013 vintage. Fresh fruit style Durif wines (H1, 2013) were characterized by red fruit and floral notes with a lower perceived bitterness, alcohol and astringency. Mature fruit style Durif wines (H5, 2013) were associated with black and dried fruits, as well as red fruit, spicy and toasted aromas with higher perceived astringency, alcohol and bitterness than fresh fruit. This corresponds to the descriptors used in literature for earlier harvested or fresh fruit style wines and later harvested or mature fruit style wines (Šuklje *et al.,* 2014; Bindon *et al.,* 2013; Deloire 2013; Deloire 2011). Neutral (in our case) wines (H2, H3, H4 of 2013) had high citation frequencies for toasted and woody notes, spice, animal, vegetable and lower citations of red and black fruit. Descriptive analysis could have added to the understanding of the intensities of these aromatic attributes for the wines at different stages.

Retrospectively, it could be observed that the 2012 low vigour wines showed the most fruity characteristics at 11, 21 and 27 days after the keypoint. It is proposed that the lack of fruitiness in the overripe or latest stages (29+ days after the keypoint) of harvest could be due to the effect of the high perceived astringency on wine fruitiness (Cliff *et al.,* 2012). The high vigour vines did not reach a plateau but showed ”rapid and continuous loading” (Deloire 2011) and did not follow the “model” of fruit sugar accumulation.

6.1.1 Practical outcomes

The detailed evaluation of the vineyard in 2012 created opportunity for learning and monitoring methods of irrigation management, canopy management, visual indicators of vine stress, visual indicators of pests and disease. An in-depth approach facilitated understanding the main indicators for differences in vigour in vineyards and how to use different indicators at different times of year.

The differences between the lateral shoot removal (LV and HV) and leaf removal treatments (FV LV and FV HV) between the commercial and experimental plots (2012) were not discussed in detail as the layout did not allow strong statistical analysis. In 2013 only leaf removal was used as a treatment to open the canopies. This was due to cost implications, as leaf removal is much more cost and time effective than lateral shoot removal. The winemakers at Fairview Winery decided the difference in wine quality was small enough to use the most financially viable canopy management practice.

6.1.2 Commercial benefits and industry

Fairview Winery has adapted its winemaking strategy as an outcome from this research project. The encouraging results inspired the Fairview team to use sequential harvesting commercially in 2013–2015 vintages. Sugar accumulation is monitored to determine which block (there are several) is most suitable for fresh and mature fruit style wines depending on sugar accumulation and Brix. The overripe stage of Durif wines has been successfully avoided where possible since 2013.

The research has been adopted and improved upon by working in the winery with Vivelys (a French company involved in vineyard and winery innovation; www.vivelys.com) on winemaking techniques such as micro-oxygenation to improve mouthfeel and reduce astringency perception. This lead to the introduction of Durif in Fairview Winery's *Goats do Roam Red* brand, a blend of 6 Rhone varieties. Through careful winemaking techniques and picking date decisions, the percentage of Durif has increased as a component of the *Goats do Roam Red* blend; every vintage adding deeper colour and structure to the blend. The research has been extended commercially to varieties other than Durif. Harvest timing and sequential harvesting principles, using sugar loading as a physiological indicator, have allowed the business to plan picking dates according to desired wine styles and become more efficient, sustainable and profitable by increasing the quality grading of the wine.

This type of research could be easily adopted by the wine industry, particularly with the support of consultants like Vivelys. More industry experience is needed in South Africa in terms of understanding fruit ripening and finding the most suitable harvest windows/sweet spots in relation to desired wine styles, and sequential harvesting in different sites as harvest timing needs to be adapted for different climates and cultivars. Grapes can be harvested at different ripeness levels for different purposes - barrel aging and/or early bottling. Sequential harvesting is an attractive solution for wineries where sequential harvesting for specific wine styles may provide practical, qualitative and logistical solutions during harvest.

6.1.3 Perspectives

Monitoring berry sugar accumulation (in addition to using berry skin colour evolution (hue angle) in white grapes; (Deloire, 2013)) provides insight into a vineyards' potential to achieve certain wine styles.

By using berry sugar accumulation and fruit fresh mass evolution, as a vineyard diagnostic method, it can quickly be seen if a vineyard is stressed (when the fresh mass decreases unexpectedly) or if there is an issue in terms of vine balance or any kind of stress experienced by the plants (when the sugar loading reaches a plateau too early at a low Brix or when sugar accumulation is very slow along the ripening process, not reaching a plateau). This additional knowledge allows a winemaker to make educated decisions on picking date, for example, choosing to harvest grapes that experienced water stress at fresh fruit, knowing they will not reach a mature fruit stage/profile. It is a method that can be used to prioritise picking of high potential blocks (reaching a plateau of berry sugar accumulation) over lower potential blocks (showing signs of stress or continuous slow sugar accumulation per berry). Used in conjunction with weather predictions, berry analysis, berry tasting and a winemaker's experience it can help optimize the picking schedule during harvest. It provides more options in difficult vintages – for example, in wet vintages some of the late varieties like Cabernet Sauvignon can be picked at fresh fruit reducing the risks of rot. However, it is important that the sampling is done correctly and the results are used in context of the vineyard (site). The method needs to be calibrated per site and cultivar.

6.1.4 Suggestions for further study

Further study on the volatile composition of sequentially harvested wines in Durif and other cultivars would be beneficial to the industry. Identification of marker compounds for wine styles, as Bindon *et al.,* (2014) suggested, would be helpful in further understanding the ripening process and evolution. Further study on the astringent sub-qualities (Ma *et al.,* 2014; Gawel *et al.,* 2000) of different Durif (and other astringent varieties) wine profiles including wines from sequentially harvested grapes would be beneficial in understanding and managing the perception of astringency.

Sensory methods for identifying small differences between similar wines (for example, Pivot profile, Napping or free sorting in combination with descriptive analysis) need to be developed for assessing wines made from sequentially harvested grapes (Heymann *et al.,* 2014*;* Sokolowsky *et al.,* 2015*;* Thuillier *et al.,* 2015*).*

6.1.5 Improvements to the project

Improvements to the 2012 vintage layout have already been noted and most improvements were implemented in the 2013 layout. Practical improvements to the project include having duplicate Tinytag™ data loggers as some experienced water damage.

The 2013 vintage could have been improved by using winemaking replicates and research over more than one vintage. Winemaking replicates were not done in 2013 as biological repeats were used to establish vineyard homogeneity and sensory analysis of 45 wines would have been impractical. The frequency of citation uses a comprehensive list of attributes for training the panel and this was used to generate the specific list for Durif. The sensory analysis could have been improved by using an attribute intensity rating in addition to the frequency citation method as the small differences between the wines did not show significant differences. However, this would be exceptionally time-consuming and descriptive analysis uses a very limited list of descriptors. The descriptors could have been reduced to match the descriptors used to describe the ripening stages, this may have given more focused results. Nonetheless, the attributes used

gave a good overall aroma profile of the wines. The use of common components specific analysis similar to Antalick *et al.,* (2015) would have provided meaningful information for the main drivers of wine style.

6.1.6 A final personal note

The journey of this research project has been interesting; exposing me to many aspects of viticulture, winemaking, research and sensory science. I've done this masters part-time while employed full-time as a winemaker at Fairview Winery. The study has broadened my outlook and knowledge and I would recommend others do the same. This research project has shown the benefits of collaboration between the wine industry and research institutes.

6.2 Bibliography

Antalick, G., Šuklje, K., Blackman, J.W., Meeks, C., Deloire, A. and Schmidtke, L.M., 2015. Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry*, 63(18) pp. 4664-72.

Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M. and Francis, I.L., 2014. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry,* 154(0), pp. 90-101.

Bindon, K., Varela, C., Kennedy, J., Holt, H. and Herderich, M., 2013. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry,* 138(2–3), pp. 1696-1705.

Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V., 2012. Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. *Analytica Chimica Acta,* 732, pp. 91-99.

Campo, E., Ballester, J., Langlois, J., Dacremont, C. and Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference,* 21(1), pp. 44-55.

Cliff, M.A., Stanich, K., Edwards, J.E. and Saucier, C.T., 2012. Adding grape seed extract to wine affects astringency and other sensory attributes. *Journal of Food Quality,* 35(4), pp. 263-271.

Deloire, A., 2011. The concept of berry sugar loading. *Wynboer,* 257, pp. 93-95.

Deloire, A., 2013. *Wine aroma and grapevine berry ripening: How to capture the complexity*. *Winetitles, Wine & Viticulture Journal 28(5).*

Deloire, A., 2013. Berry ripening and wine aroma. *Practical Winery and Vineyard,* pp. 64-67.

Eichhorn, K. and Lorenz, D., 1977. Phenological development stages of the grape vine. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes,* **29**(8), pp. 119-120.

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J., Moutounet, M. and Cheynier, V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry,* 54(19), pp. 7331-7338.

Gawel, R., Oberholster, A. and Francis, I.L., 2000. A Mouth-feel Wheel: terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research,* **6**(3), pp. 203-207.

Gil, M., Quirós, M., Fort, F., Morales, P., Gonzalez, R., Canals, J. and Zamora, F., 2015. Influence of Grape Maturity and Maceration Length on Polysaccharide Composition of Cabernet Sauvignon Red Wines. *American Journal of Enology and Viticulture,* .

Heymann, H., Hopfer, H. and Bershaw, D., 2014. An Exploration of the Perception of Minerality in White Wines by Projective Mapping and Descriptive Analysis. *Journal of Sensory Studies,* 29(1), pp. 1-13.

Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y. and Li, H., 2014. A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science & Technology,* 40(1), pp. 6-19.

Nell, M., 2015. *Sensory characterisation of several red cultivar (Vitis vinifera L.) wines, using berry sugar accumulation as a physiological indicator and sequential harvest,* .

Pérez-Magariño, S. and González-San José, M.L., 2006. Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chemistry,* 96(2), pp. 197-208.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and DUBOURDIEU, D., 2000. Handbook of enology. The chemistry of wine and stabilisation and treatments, vol. 2.

Sokolowsky, M., Rosenberger, A. and Fischer, U., 2015. Sensory impact of skin contact on white wines characterized by descriptive analysis, time–intensity analysis and temporal dominance of sensations analysis. *Food Quality and Preference,* 39, pp. 285-297.

Šuklje, K., Antalick, G., Meeks, C., Blackman, J.W., Deloire, A. and Schmidtke, L.M., November 2014. Optimising harvest date through use of an integrated compositional and sensory model: a proposed approach for better understanding of ripening of autochthonous varieties? *2nd International Oneoviti symposium* November 2014.

Thuillier, B., Valentin, D., Marchal, R. and Dacremont, C., 2015. Pivot© profile: A new descriptive method based on free description. *Food Quality and Preference,* 42, pp. 66-77.

Zerihun, A., Lanyon, D. and Gibberd, M., 2010. Vine vigour effects on leaf gas exchange and resource utilisation. *Australian Journal of Grape and Wine Research,* 16(1), pp. 237-242.

APPENDIX A

Table A1: Number of bunches per vine and yield (kg/vine) for the panels, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Panel number	Average number of bunches per vine	Average yield per vine (kg)	Mean mass per bunch (g)
1	28.7 ± 1.76 [ab]	3.86 ± 0.47 [b]	136.0 ± 11.69 [b]
2	27.3 ± 1.73 [b]	4.32 ± 0.46 [ab]	155.9 ± 11.46 [ab]
3	27.2 ± 1.73 [b]	4.41 ± 0.46 [ab]	162.5 ± 11.46 [ab]
4	28.6 ± 1.73 [ab]	4.02 ± 0.46 [b]	140.2 ± 11.30 [b]
5	30.9 ± 1.90 [ab]	4.92 ± 0.52 [ab]	159.4 ± 12.76 [ab]
6	28.9 ± 1.67 [ab]	4.59 ± 0.45 [ab]	157.6 ± 11.11 [ab]
7	32.8 ± 1.87 [a]	4.92 ± 0.51 [ab]	149.9 ± 12.55 [ab]
8	27.4 ± 1.67 [b]	3.78 ± 0.45 [b]	137.0 ± 11.11 [b]
9	30.6 ± 1.76 [ab]	5.61 ± 0.47 [ab]	177.6 ± 11.69 [a]

Table A2: Number of bunches per vine and yield (kg/vine) for biological repeats, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Biological repeat	Mean number of bunches per vine	Mean mass of grapes per vine (kg)	Mean mass per bunch (g
Blue	30.54 ± 1.65 [a]	4.71 ± 0.50 [a]	152.24 ± 11.98 [a]
Green	28.64 ± 1.62 [a]	4.47 ± 0.49 [a]	153.74 ± 11.78 [a]
Red	28.06 ± 1.61 [a]	4.25 ± 0.49 [a]	151.81 ± 11.69 [a]

Table A3: Main and lateral shoot length per panel, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Panel	Main shoot length (cm)	Number of lateral shoots	Lateral shoot length (cm)
1	92.25 ±11.89 [b]	3.25 ±1.04 [b]	5.75 ±9.60 [b]
2	109.67 ±13.72 [ab]	6.00 ±1.20 [ab]	11.50 ±11.08 [ab]
3	111.67 ±13.72 [ab]	5.67 ±1.20 [ab]	13.67 ±11.08 [ab]
4	93.00 ±11.89 [b]	5.50 ±1.04 [ab]	19.50 ±9.60 [ab]
5	107.00 ±13.72 [ab]	5.33 ±1.20 [ab]	42.00 ±11.08 [a]
6	88.00 ±13.72 [b]	5.00 ±1.20 [ab]	20.67 ±11.08 [ab]
7	90.38 ±11.89 [b]	6.25 ±1.04 [ab]	15.63 ±9.60 [ab]
8	119.00 ±13.72 [ab]	7.00 ±1.20 [a]	37.00 ±11.08 [a]
9	136.33 ±13.72 [a]	6.67 ±1.20 [a]	40.00 ±11.08 [a]

Table A4: Main and lateral shoot length per biological repeat, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Biological Repeat	Main shoot length (cm)	Number of lateral shoots	Lateral shoot length (cm)
Blue	103.30 ± 7.95 [a]	4.8 ± 0.63 [a]	9.85 ± 6.13 [b]
Green	95.70 ± 7.95 [a]	5.3 ± 0.63 [a]	26.60 ± 6.13 [ab]
Red	112.75 ± 7.95 [a]	6.6 ± 0.63 [a]	29.35 ± 6.13 [a]

Table A5: Number of leaves and leaf area per panel, expressed as average ± SEM. Statistically significant differences are expressed using letters

Panel	Number of leaves (main shoot)	Main shoot leaf area	Number of leaves (lateral shoot)	Lateral shoot leaf area
1	10.50 ± 1.26 [b]	871.08 ± 175.70 [b]	5.50 ± 2.00 [c]	145.78 ± 88.40 [c]
2	13.00 ± 1.45 [b]	1089.80 ± 202.88 [ab]	9.00 ± 2.31 [cb]	226.77 ± 102.08 [cb]
3	12.00 ± 1.45 [b]	957.93 ± 202.88 [ab]	9.00 ± 2.31 [cb]	277.40 ± 102.08 [cb]
4	9.75 ± 1.26 [b]	806.55 ± 175.70 [b]	10.50 ± 2.00 [ac]	404.80 ± 88.40 [cb]
5	12.00 ± 1.45 [b]	954.83 ± 202.88 [ab]	9.00 ± 2.31 [cb]	285.57 ± 102.08 [cb]
6	10.67 ± 1.45 [b]	964.43 ± 202.88 [ab]	9.67 ± 2.31 [ac]	296.10 ± 102.08 [cb]
7	11.50 ± 1.26 [b]	826.50 ± 175.70 [b]	8.75 ± 2.00 [cb]	231.95 ± 88.40 [cb]
8	13.67 ± 1.45 [ab]	1029.93 ± 202.88 [ab]	16.33 ± 2.31 [a]	698.37 ± 102.08 [a]
9	17.33 ± 1.45 [a]	1442.00 ± 202.88 [a]	13.33 ± 2.31 [ab]	508.03 ± 102.08 [ab]

Table A6: Number of leaves and leaf area per biological repeat, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Biological repeat	Main shoot length (cm)	Number of lateral shoots	Lateral shoot length (cm)	Main shoot leaf area	Number of leaves (lateral shoot)	Lateral shoot leaf area
Blue	103.30 ± 7.95 a	4.8 ± 0.63 a	9.85 ± 6.13 b	962.75 ± 112.03 a	7.60 ± 1.32 b	209.56 ± 63.94 b
Green	95.70 ± 7.95 a	5.3 ± 0.63 a	26.60 ± 6.13 ab	898.40 ± 112.03 a	9.80 ± 1.32 ab	336.42 ± 63.94 ab
Red	112.75 ± 7.95 a	6.6 ± 0.63 a	29.35 ± 6.13 a	1072.18 ± 112.03 a	12.40 ± 1.32 a	454.70 ± 63.94 a

Table A7: Pruning and bunch mass per panel, expressed as average ± SEM. Statistically significant differences are expressed using letters.

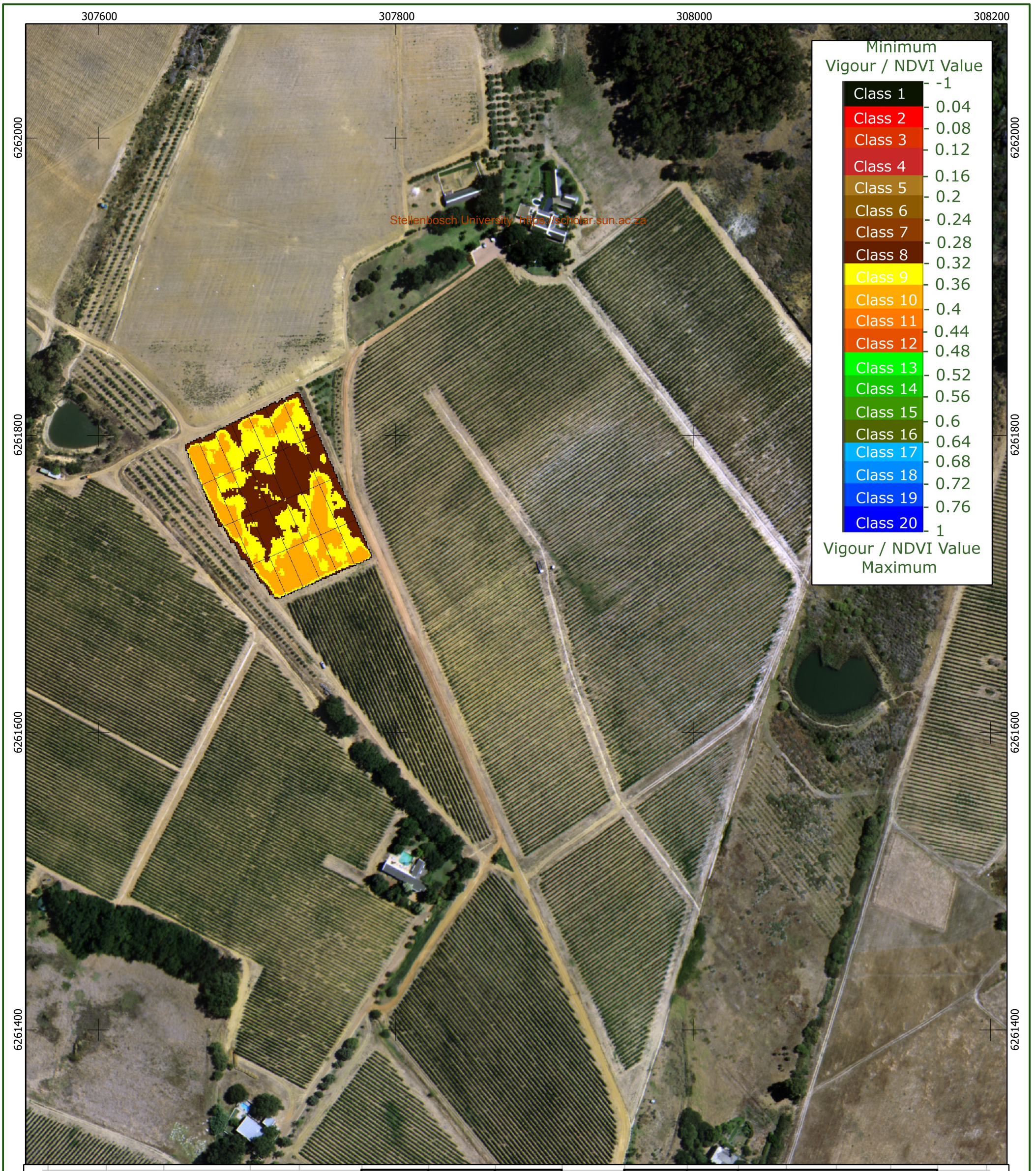
Panel	Mean number of canes per vine	Mean mass of canes per vine (kg)	Mean number of bunches per vine	Mean mass of grapes per vine (kg)	Mean mass per bunch (g)	Cane mass:yield / vine
1	24.83 ± 1.70 a	0.75 ± 0.10 ab	30.42 ± 2.79 ab	4.04 ± 0.77 b	131.58 ± 18.12 b	0.20 ± 0.02 a
2	18.75 ± 1.61 cb	0.52 ± 0.10 b	25.50 ± 2.64 b	3.70 ± 0.73 b	142.13 ± 17.19 ab	0.15 ± 0.02 ab
3	20.30 ± 1.56 ac	0.73 ± 0.09 ab	28.50 ± 2.55 ab	3.96 ± 0.71 ab	138.55 ± 16.61 ab	0.20 ± 0.02 ab
4	21.13 ± 1.61 ac	0.78 ± 0.10 ab	30.25 ± 2.64 ab	4.74 ± 0.73 ab	159.00 ± 17.19 ab	0.17 ± 0.02 ab
5	19.38 ± 1.61 cb	0.76 ± 0.10 ab	32.63 ± 2.64 ab	4.75 ± 0.73 ab	146.88 ± 17.19 ab	0.17 ± 0.02 ab
6	20.38 ± 2.08 ac	0.65 ± 0.12 ab	28.75 ± 3.41 ab	4.71 ± 0.95 ab	161.25 ± 22.19 ab	0.15 ± 0.03 b
7	22.96 ± 1.42 ab	0.78 ± 0.08 ab	34.63 ± 2.33 a	5.41 ± 0.65 ab	153.29 ± 15.16 ab	0.15 ± 0.02 b
8	17.63 ± 1.61 c	0.57 ± 0.10 b	28.25 ± 2.64 ab	3.85 ± 0.73 b	136.63 ± 17.19 ab	0.15 ± 0.02 ab
9	22.50 ± 1.56 ac	0.88 ± 0.09 a	32.35 ± 2.55 ab	5.82 ± 0.71 a	177.00 ± 16.61 a	0.15 ± 0.02 b

Table A8: Pruning and bunch mass per biological repeat, expressed as average ± SEM. Statistically significant differences are expressed using letters.

Biological repeat	Number of canes per vine	Mass of canes per vine (kg)	Number of bunches per vine	Mass of grapes per vine (kg)	Cane mass:yield/vine	Mass per bunch (g)
Blue	22.06 ± 1.05 [a]	0.70 ± 0.06 [a]	30.89 ± 1.61 [a]	4.57 ± 0.46 [a]	0.16 ± 0.01 [a]	147.03 ± 10.40 [a]
Green	21.12 ± 0.97 [a]	0.75 ± 0.06 [a]	29.96 ± 1.49 [a]	4.63 ± 0.43 [a]	0.17 ± 0.01 [a]	152.79 ± 9.63 [a]
Red	20.21 ± 0.97 [a]	0.73 ± 0.06 [a]	30.90 ± 1.49 [a]	4.79 ± 0.43 [a]	0.16 ± 0.01 [a]	153.40 ± 9.63 [a]

Table A9: NDVI Classes

Mean	Range		Colour	Classes
< 0.04	-1.00	0.04	Black	Class 1
0.06	0.04	0.08	Red 1	Class 2
0.10	0.08	0.12	Red 2	Class 3
0.14	0.12	0.16	Red 3	Class 4
0.18	0.16	0.20	Brown 1	Class 5
0.22	0.20	0.24	Brown 2	Class 6
0.26	0.24	0.28	Brown 3	Class 7
0.30	0.28	0.32	Brown 4	Class 8
0.34	0.32	0.36	Yellow 1	Class 9
0.38	0.36	0.40	Yellow 2	Class 10
0.42	0.40	0.44	Yellow 3	Class 11
0.46	0.44	0.48	Yellow 4	Class 12
0.50	0.48	0.52	Green 1	Class 13
0.54	0.52	0.56	Green 2	Class 14
0.58	0.56	0.60	Green 3	Class 15
0.62	0.60	0.64	Green 4	Class 16
0.66	0.64	0.68	Blue 1	Class 17
0.70	0.68	0.72	Blue 2	Class 18
0.74	0.72	0.76	Blue 3	Class 19
> 0.76	0.76	1.00	Blue 4	Class 20



Block	Abbrev.	Grape Cultivar	Net	Training	Separation Rows	Separation Plants	Separation Posts	Class	Pixels Quantity	Area (has)	NDVI Min.	NDVI Max.	NDVI Mean.	NDVI StD.	NDVI CV
03	**du**	**Durif**	No	Trellis	2.50	1.25		Totales	9,860	0.99	0.147	0.470	0.340	0.036	10.56
								8	3,345	0.33	0.147	0.321	0.303	0.016	5.41
								9	3,605	0.36	0.321	0.361	0.340	0.012	3.43
								10	2,910	0.29	0.361	0.470	0.384	0.020	5.23

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Fairview

Minor Unit

Field: 03

Projection: Transverse Mercator
Datum: WGS84 UTM34 South
Scale: 1 : 2500

50 0 50 meters

FOUNDED 1693
FAIRVIEW

Project No.: L04528 Map No.: L04528/20130210/09 Date: Feburary 2013

APPENDIX B.1

JUDGE nr:

Wine Code: ☐

1- Odours Description

AROMATIC DESCRIPTORS LIST

☐ **FRUITY**

☐ **WHITE FRUITS**
- ☐ Pear
- ☐ Green Apple

☐ **YELLOW FRUITS**
- ☐ Peach

☐ **CITRUS**
- ☐ Lemon
- ☐ Orange

☐ **RED FRUITS**
- ☐ Cherry
- ☐ Strawberry
- ☐ Raspberry
- ☐ Redcurrant

☐ **BLACK FRUITS**
- ☐ Blackcurrant
- ☐ Blueberry
- ☐ Blackberry

☐ **DRIED FRUITS**
- ☐ Date
- ☐ Dried Fig
- ☐ Prune
- ☐ Dried Apricot
- ☐ Raisin

☐ **HARD FRUITS (NUTS)**
- ☐ Almond

☐ **TROPICAL FRUITS**
- ☐ Pineapple
- ☐ Passion Fruit
- ☐ Guava

☐ **Cider**

☐ **Jammy**

☐ **Glazed / Crystallized Fruit**

☐ **FLORAL**
- ☐ Geranium
- ☐ Lilac
- ☐ Violet
- ☐ Honey

☐ **VEGETATIVE**

☐ **VEGETABLES**
- ☐ Asparagus
- ☐ Cabbage
- ☐ Green Beans
- ☐ Olive

☐ **Hay / Dried Grass**

☐ **Herbaceous / Green Grass**

☐ **Eucalyptus**

☐ **Mint**

☐ **Tobacco**

☐ **SPICE**
- ☐ Cinnamon
- ☐ Clove
- ☐ Juniper
- ☐ Bay Leaf / Laurel
- ☐ Nutmeg
- ☐ Black Pepper

☐ **ANIMAL**
- ☐ Leather
- ☐ Smoked
- ☐ Meat Stock
- ☐ Horsy / Sweaty

☐ **TOASTED / WOOD**

☐ **TOASTED**
- ☐ Caramel
- ☐ Roasted Coffee
- ☐ Toasted Bread
- ☐ Vanilla
- ☐ Chocolate
- ☐ Toffee

☐ **WOODY**
- ☐ Woody/ Planky
- ☐ Toasted / Smoked Wood

☐ **FOREST FLOOR**
- ☐ Mushroom
- ☐ Humus / Earthy

☐ **OTHERS**
- ☐ Alcohol
- ☐ Lactic
- ☐ Chalky
- ☐ Rubber
- ☐ Sulphur
- ☐ Solvent / Chemical

☐ Other choice (specifiec _____

Choose the most relevant descriptors on the list below by ticking the corresponding box, 5 descriptors maximum

2- Intensity rating of Taste

	Absent - 0	Very low - 1	Low - 2	Medium - 3	High - 4	Very high - 5
Sweetness	☐	☐	☐	☐	☐	☐
Sourness	☐	☐	☐	☐	☐	☐
Bitterness	☐	☐	☐	☐	☐	☐

Intensity rating of Mouthfeel

	Absent - 0	Very low - 1	Low - 2	Medium - 3	High - 4	Very high - 5
Astringency	☐	☐	☐	☐	☐	☐
Alcohol	☐	☐	☐	☐	☐	☐

Table B.1 Standards used for sensory evaluation

Subfamily / Family	Descriptor	Odour reference and quantity
Fruit		
White fruit	Green apple	2 cm3 fresh green apple cut just before the session
	Pear	2 cm3 of canned pear + 10 mL distilled water
	Quince	3 spoons of Quince marmalade "Ann's kitchen"
Yellow fruit	Apricot	20 mL apricot juice "Liquidfruit" + 1/4 of canned fruit "Rhodes "
	Melon	fresh melon cut in pieces (15 min prior to the session)
	Peach	3 cm3 canned peach "Koo" + 3 cm3 fresh peach + 5 mL distilled water
Citrus	Bergamot	Solution standard "Ferminich", 2 drops on a cotton disk
	Grapefruit	3 cm3 of fresh fruit (pulp + flesh)
	Lemon	1 drop essence "Vahine" on a cotton disk
	Orange	1 drop essence "Robertsons " on a cotton disk
Red fruit	Cherry	Solution of 5 mL "Vedrenne" syrup + 15 mL distilled water
	Raspberry	1 big spoon Nappage "Vahine"
	Redcurrant	Solution 5 frozen berries Hillcres t + 10 mL distilled water
	Strawberry	1/2 of a fresh strawberry
Black fruit	Blackberry	Solution 5mL "Vedrenne" syrup + 15 mL distilled water
	Blackcurrant	Solution of 5 frozen berries "Hillcres t" + 10 mL distilled water
	Blueberry	2 spoons Blueberry sauce "St Dal four"
Dried fruit	Date	1 date "Safari " cut in pieces
	Dried	apricot 3 half of dried apricots "Freshers"
	Dried	Fig 1 sun dried fig "Freshers "in pieces
	Prune	1 dried prune "Safari " cut in pieces
Hard fruit (nutty)	Almond	Solution 10 drops of almond essence "Vahine" + 10 mL distilled water
	Hazelnut	Solution 2 little spatulas of "Nutella"
	Walnut	An oral comment was provided to panellists
Tropical fruit	Banana	1 cm3 ripe banana +10 mL distilled water
	Coconut	6g dry coconut "Imbo" + 20 mL of hot water
	Goose berry	3 fresh goose berries cut in pieces
	Guava	20 mL guava juice "Darling"
	Litchi	1 dried litchi rehydrated
	Mango	2 cm3 canned mango +1 mL mango juice "Darling" + 5 mL distilled water
	Passion fruit	1/4 of the pulp from a fresh passion fruit
	Pineapple	2 cm3 of a fresh pineapple
Other fruit	Oxidized Apple	2 cm3 fresh apple, leave it for a while to get the oxidation
	Bitter almond	2 drops of bitter almond essence "Vahine"
	Cider	Solution 20 mL dry cider "Hunter's "
	Crystallized fruit	3 cm3 pieces of crystallized fruit "Moir's " + 10 mL boiled water

	Kirschy	An oral comment was provided to panellists
	Muscat	An oral comment was provided to panellists
Vegetative		
Vegetables	Artichoke	1/2 piece of a can
	Asparagus	10 mL water from a can "Food Lover's signature"
	Cabbage	Cooked fresh cabbage
	Celery	2 cm3 fresh celery
	Green bean	10 mL water from a can "Koo"
	Green pepper	4 thongs of a fresh green pepper cut in pieces
	Olive	1 olive + 10 mL water from a can
Other vegetative	Eucalyptus	1 drop solution of Eucalyptol
	Hay / dried grass	Finely cut hay - get from pet shop
	Herbaceous Fresh grass	A half bottle of fresh grass
	Lemon grass	1 cm3 cut in little pieces of fresh lemon grass
	Mint	2 fresh smashed mint leaves
	Tobacco	Dried tobacco from 2 cigarettes
	Tomato leaf	Green leaves of cherry tomato + stem
Floral		
	Acacia	An oral comment was provided to panellists
	Camomile	1 tea bag into 30 mL of hot water during 10 min
	Geranium	1 drop solution "Aux parfums de Gras se" on a cotton disk
	Honey	1 little spoon of honey + 10 mL of hot water
	Honeysuckle	2 drops solution "Ferminich" on a cotton disk
	Jasmin	1 drop of solution "Aux parfums de Gras se" on a cotton disk
	Lilac	2 drops solution "Ferminich" on a cotton disk
	Linden Tree Flower	2 drops solution "Aux parfums de Gras se" on a cotton disk
	Orange Blossom	2 drops solution "Ferminich" on a cotton disk
	Rose	Solution of 1 mL of rose water +10 mL distilled water
	Violet	Solution 2 mL of "Vedrenne" syrup + 4 mL distilled water
SPICE		
	Ani seed/Fennel	10 drops "Carrefour" aniseed syrup
	Bay leaf / Laurel	1 cut dried bay leaf
	Black pepper	2g whole berries black pepper crushed, "Chekers's Choice"
	Cinnamon	0,05g cinnamon powder, "Chekers's Choice"
	Clove	0,05g "Robertsons " clove powder
	Curry	1 small spatula of curry powder
	Ginger	1g of ginger powder "Robertsons "
	Juniper	2 crushed berries of Juniper
	Liquorice	2 cm of "Mister sweet"
	Nutmeg	0,03g Nutmeg powder "Robertsons "
	Thyme	1 spatula of "Robertsons " dried thyme
	White pepper	2g "Robertsons " white pepper in powder + 5 mL distilled water

ANIMAL		
	Cat urine	An oral comment was provided to panellists
	Horsy / sweaty	An oral comment was provided to panellists
	Leather	Leather pieces were passed among panellists
	Meat Stock	Solution 25 g of beef stock "Ina Paarman's kitchen" + 10 mL of diluted Beefy Borril
	Musk / Civet	An oral comment was provided to panellists
	Smoked	1 little spoon of BBQ sauce Jack Daniels
	Wet dog	wet dog hair
FOREST FLOOR		
	Humus / Earthy	wet earth (a half bottle)
	Mouldy	An oral comment was provided to panellists
	Mushroom	Solution 1/2 fresh mushroom cut in pieces + 10 mL distilled water
TOASTED / WOOD		
Toasted	Caramel	Solution 1 big spoon "Vahine" caramel + 5 mL hot water
	Chocolate	1 spoon Nappage "Vahine" (sauce)
	Roasted Coffee	Solution 1 g instant coffee "Jacobs " + 10 mL hot water
	Toasted bread	1x1 cm toasted bread
	Toffee	Solution 1 and a half toffee sweet + 5 mL hot water, to melt in the microwaves
	Vanilla	1/2 teaspoon "Vahine" vanilla essence
Woody	Burnt wood	Pieces of burnt chips wood
	Toasted / Smoked wood	5g toasted wood
	Woody / Planky	5g new wood
OTHER		
	Alcohol	5 mL of alcohol at 96%
	Butter	2 cm3 of fresh butter
	Carton / dust	Carton / dust Pieces carton + a few drops of water
	Chalky	Chalky pieces were passed among panellists
	Chemical strawberry	3 drops of strawberry flavour "IFF" on a cotton disk
	Iodine / Salty	1/4 crushed oyster shell
	Lactic	20 mL of fresh pas teuri zed cream "Darling"
	Mineral / Flinty	An oral comment was provided to panellists
	Rubber	1 cm of a rubber pipe, to warm up in the microwaves
	Solvent / Chemical	Ethyl acetate 200 microL
	Stuffy / Fusty smell	An oral comment was provided to panellists
	Sulphur	Solution 300 microL SO2 at 15% + 15 mL distilled water
	Tar	1 little spatula of Creosote
	Wet mop	Pieces of wet mop
	Yeast	20 mL rehydrated yeasts from the wine industry

APPENDIX B.2

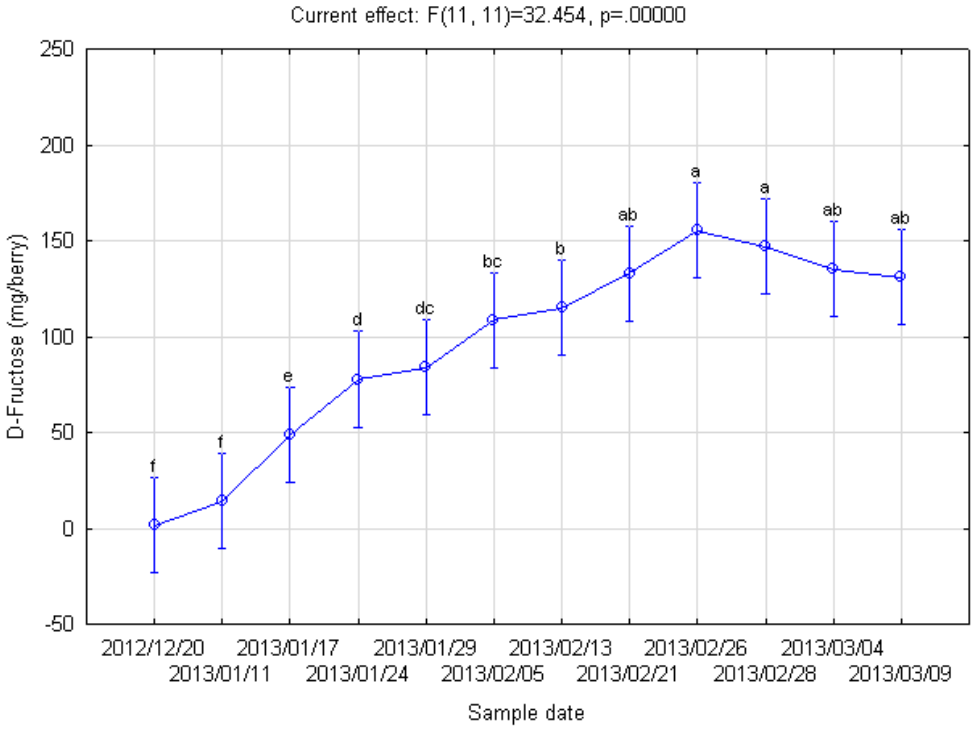

Figure B1: D-Fructose accumulation per berry

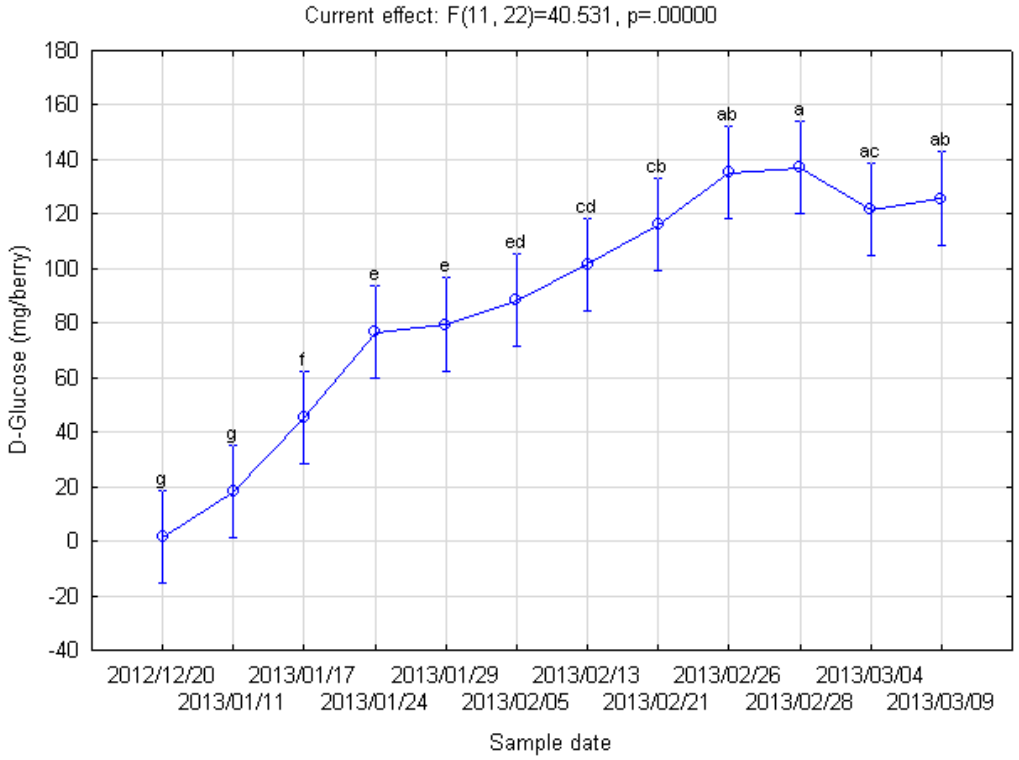

Figure B2: D-Glucose accumulation per berry

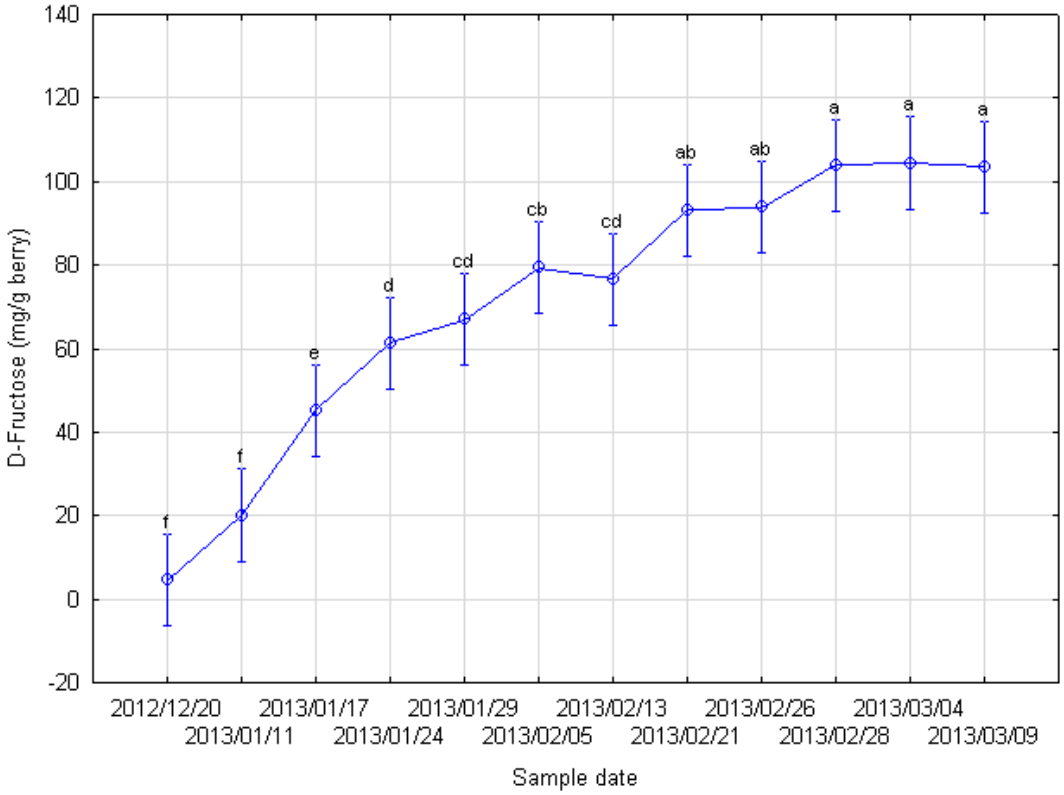

Figure B3: D-Fructose accumulation per gram berry

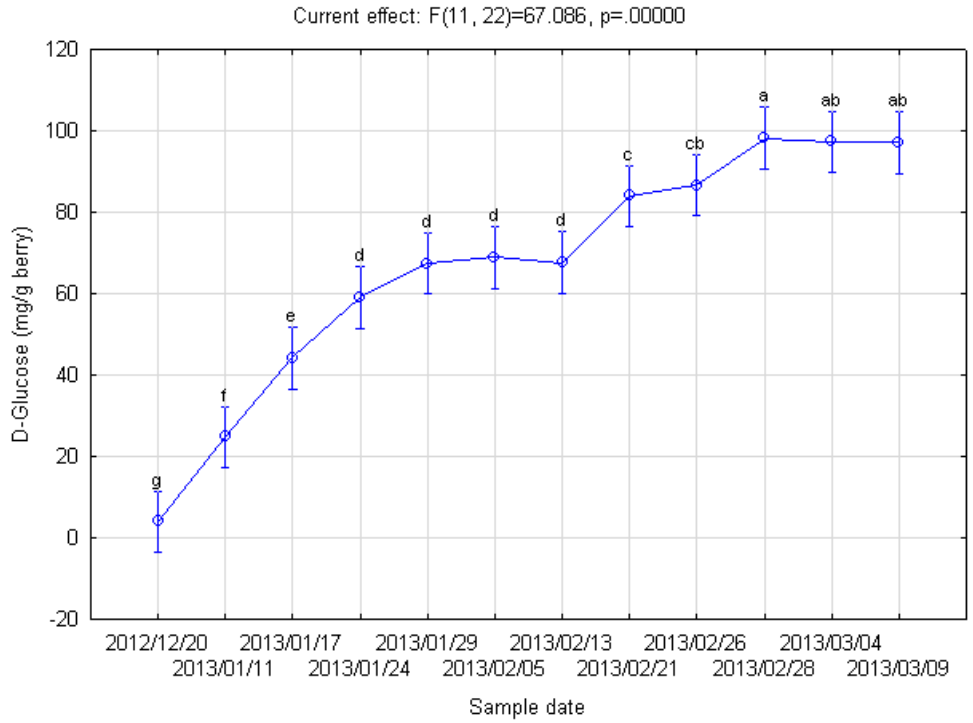

Figure B4: D-Glucose accumulation per gram berry

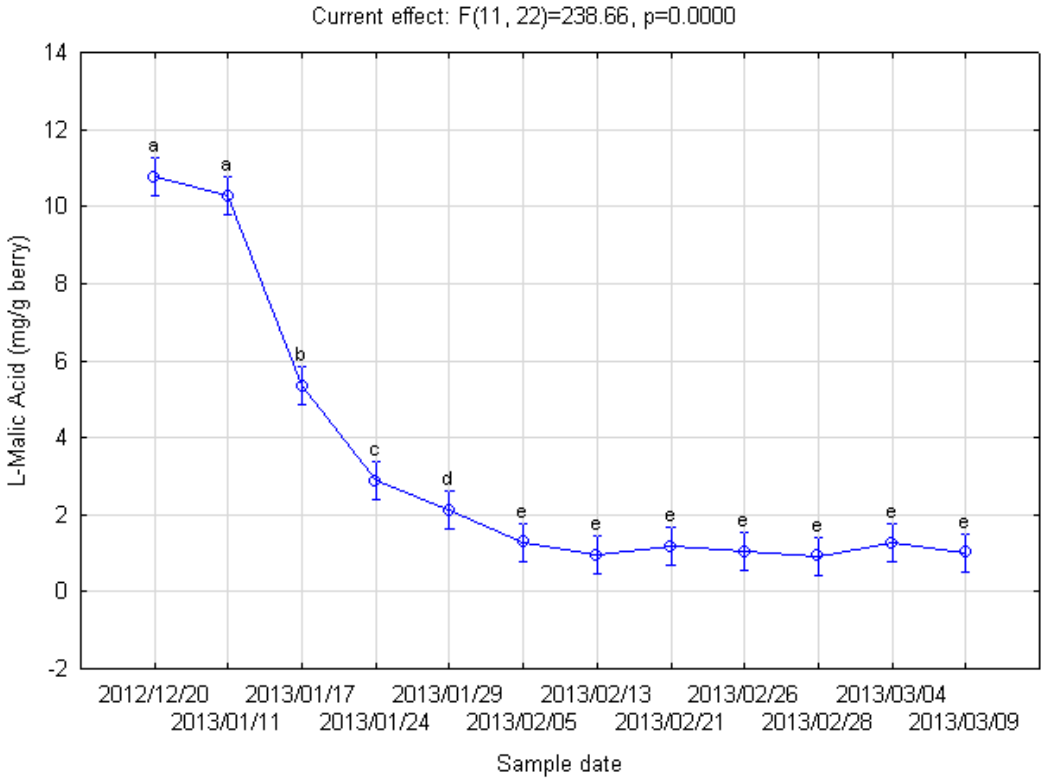

Figure B5: Degradation of L-Malic acid per gram berry

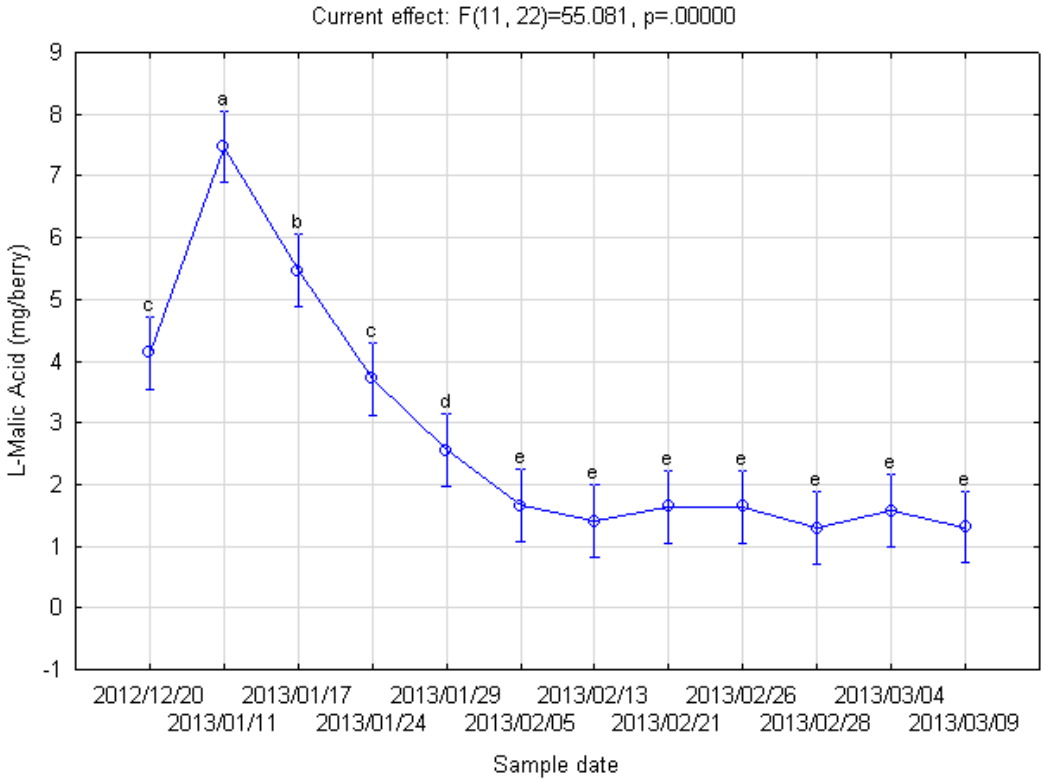

Figure B6: Degradation of L-Malic acid per berry

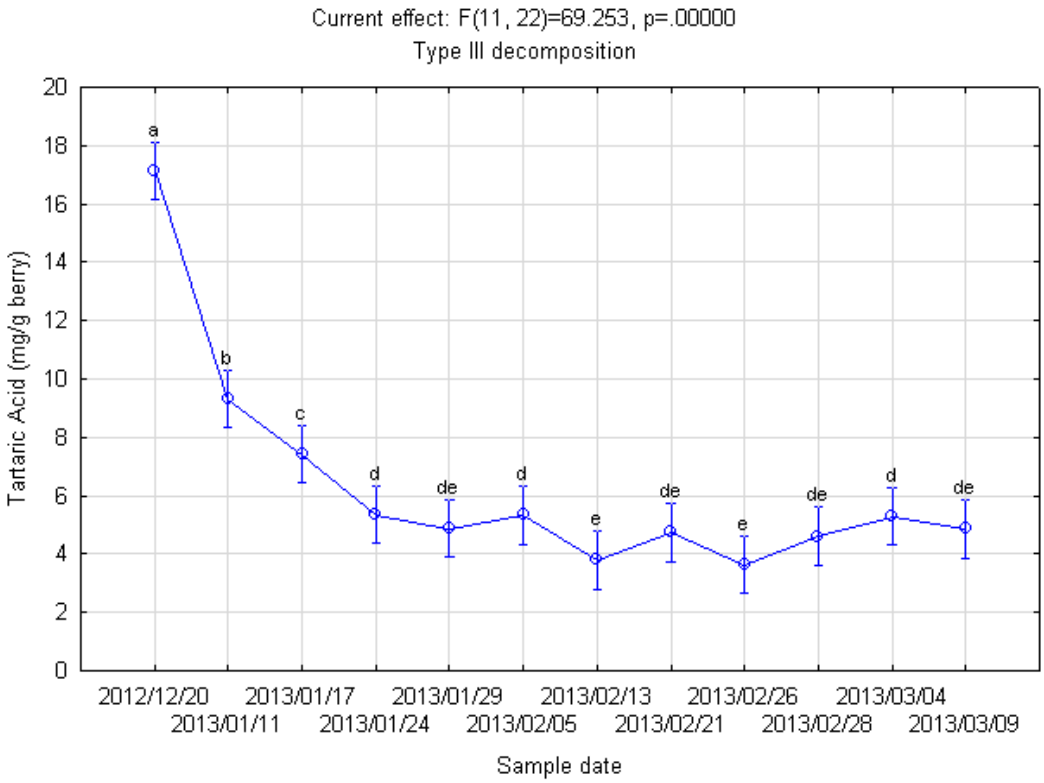

Figure B7: Degradation of Tartaric acid per gram berry

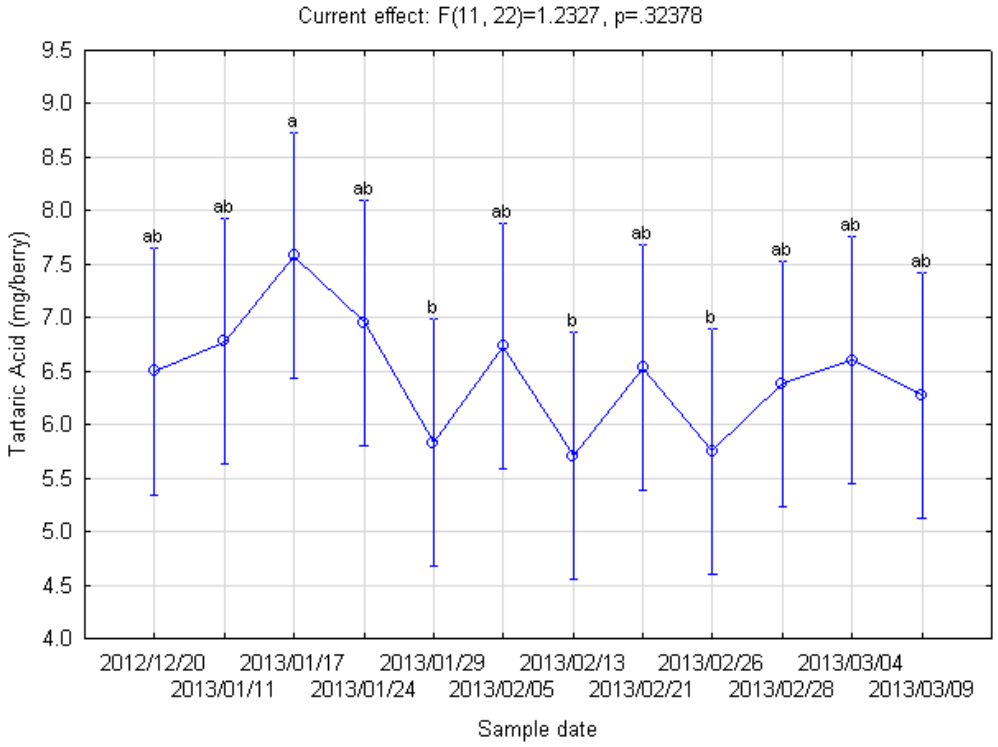

Figure B8: Tartaric acid per berry

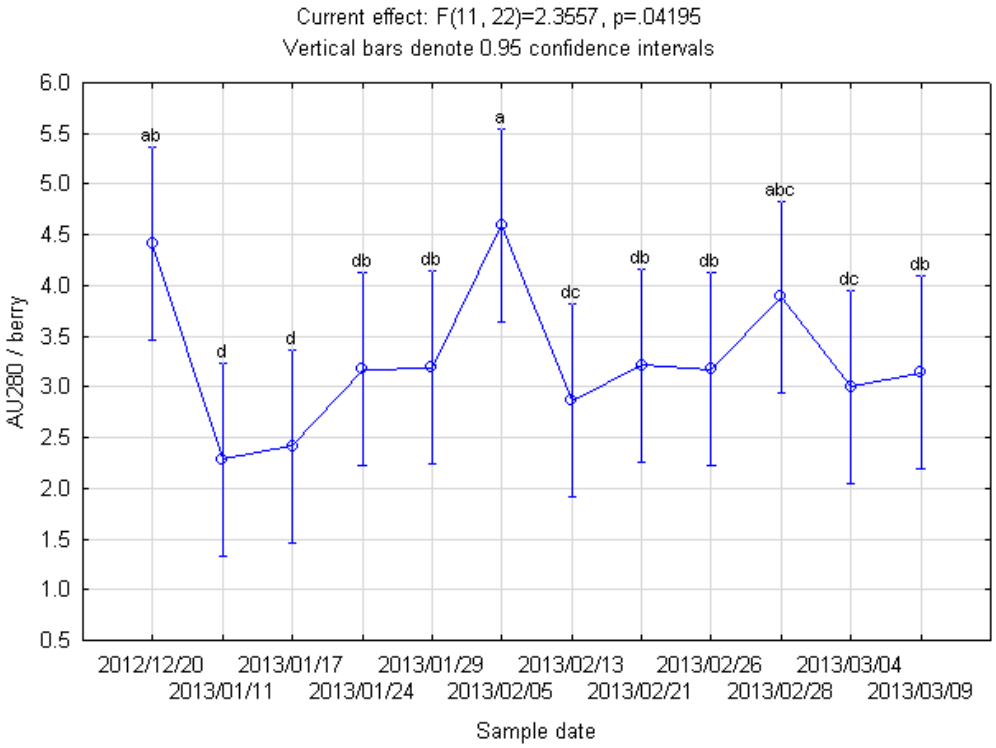

Figure B9: AU 280 per berry

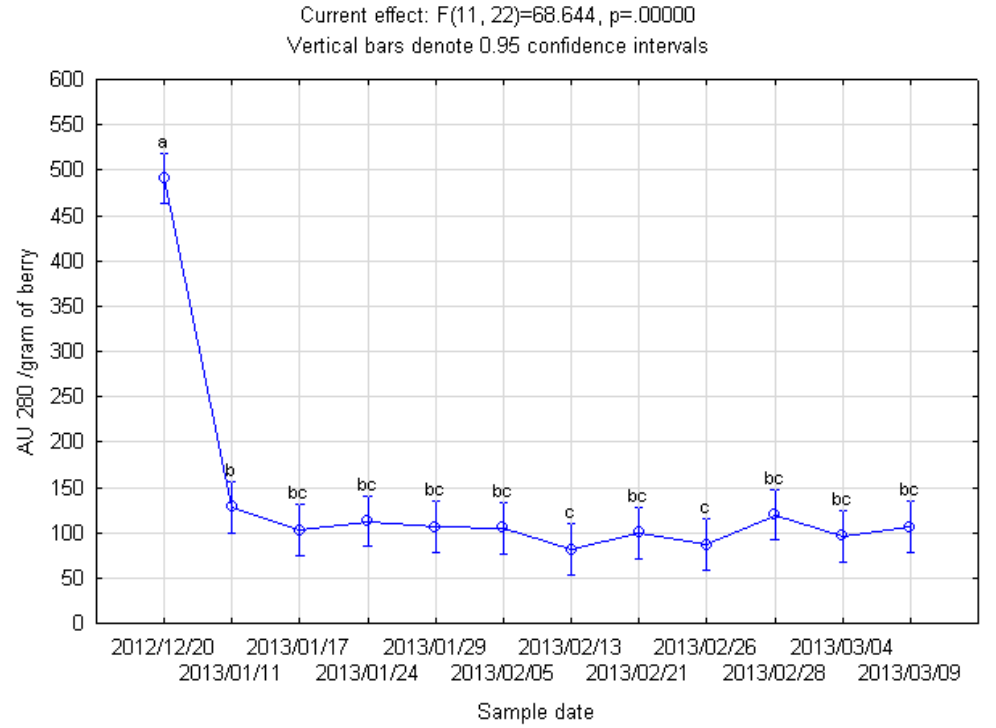

Figure B10: AU 280 per gram of berry

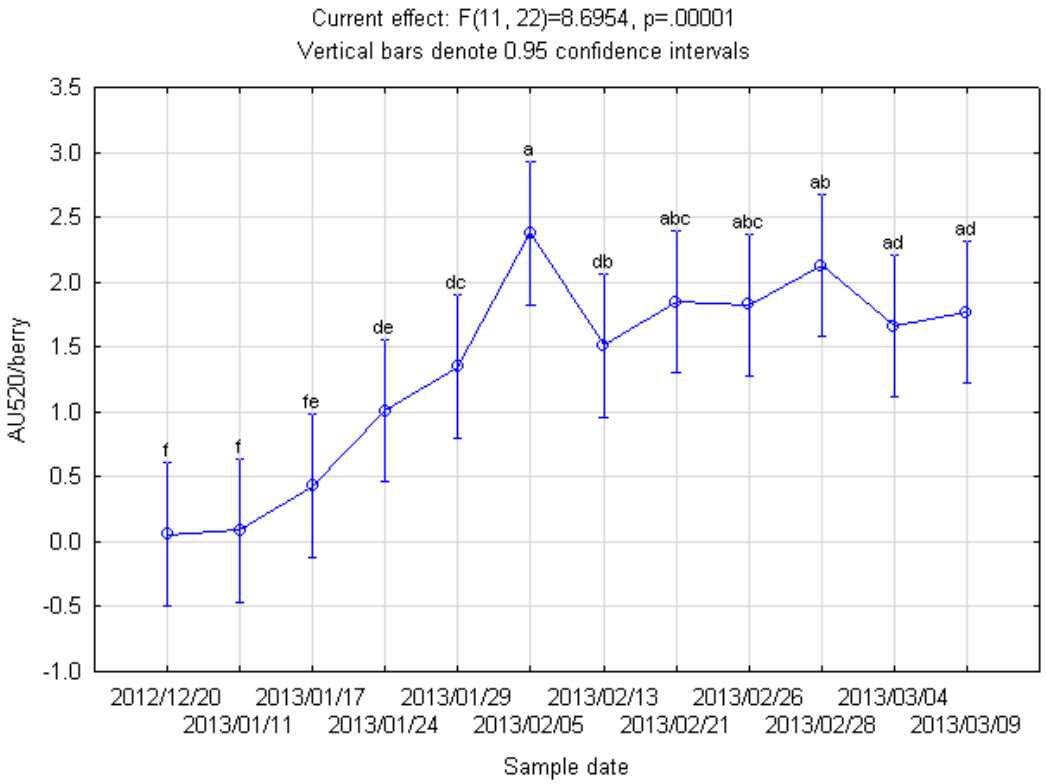

Figure B11: AU520 per berry

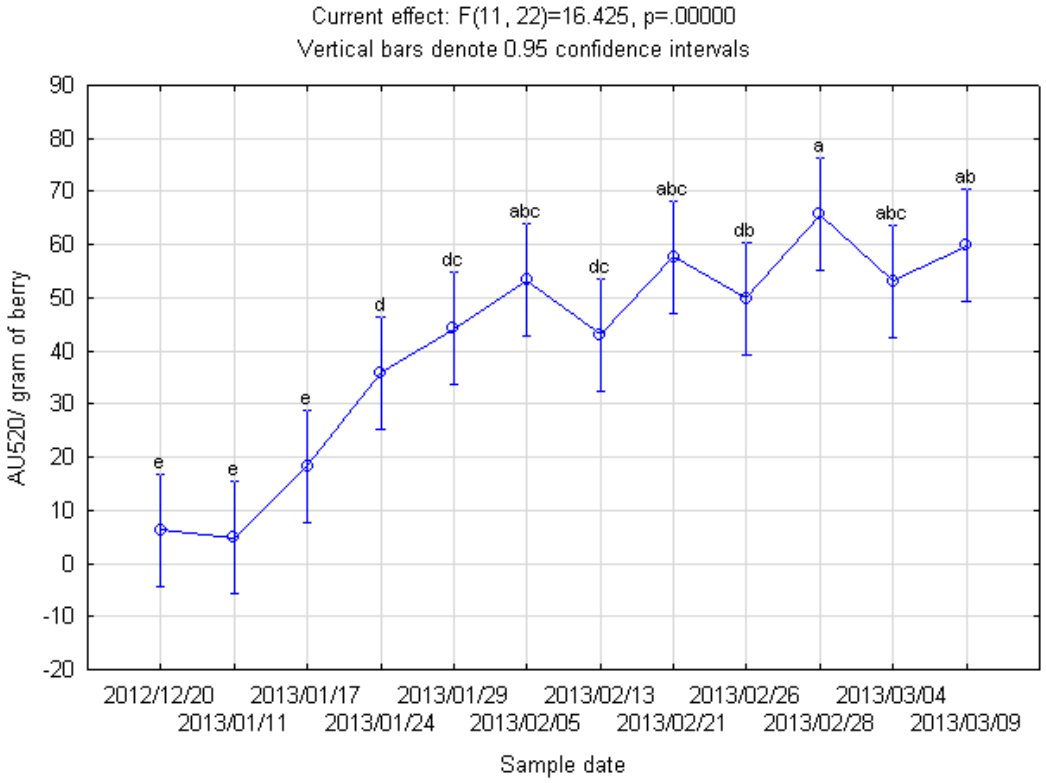

Figure B12: AU 520 per gram of berry

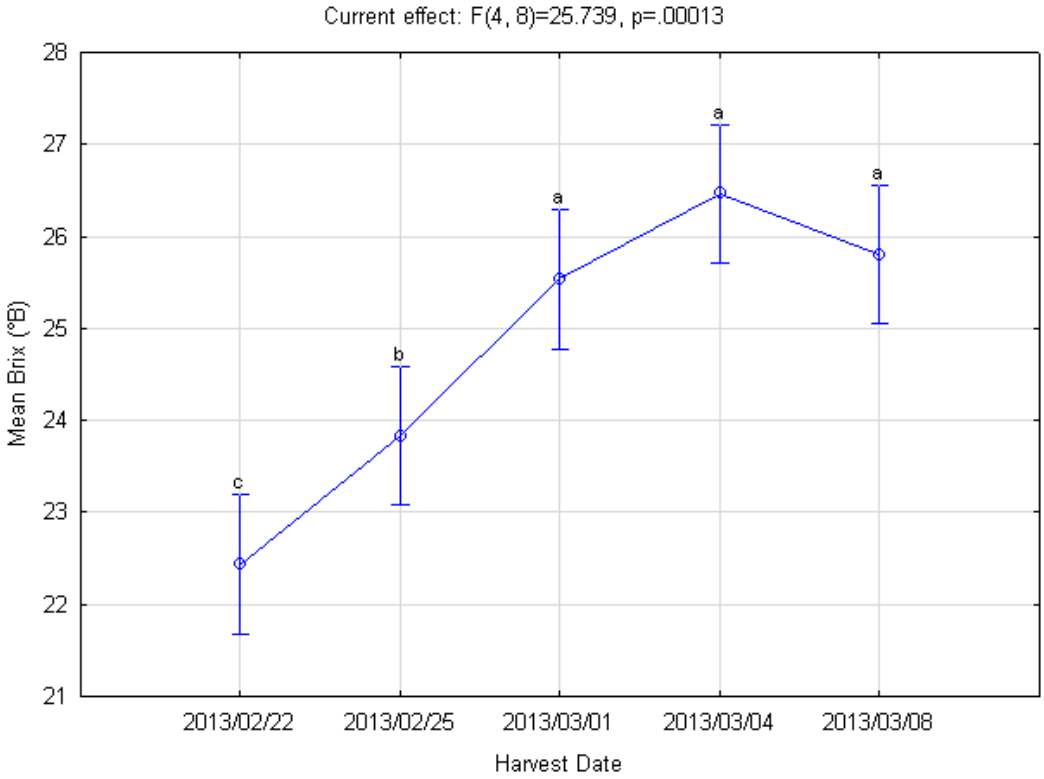

Figure B13: Mean Brix per sequential harvest date

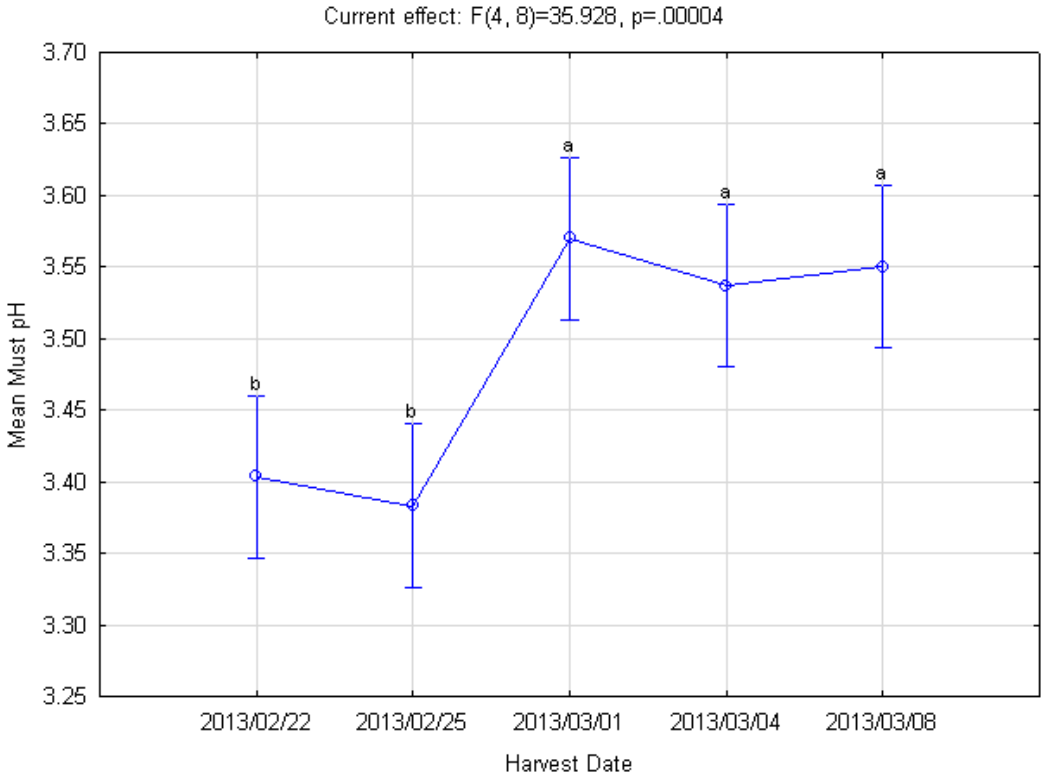

Figure B14: Mean must pH per harvest date

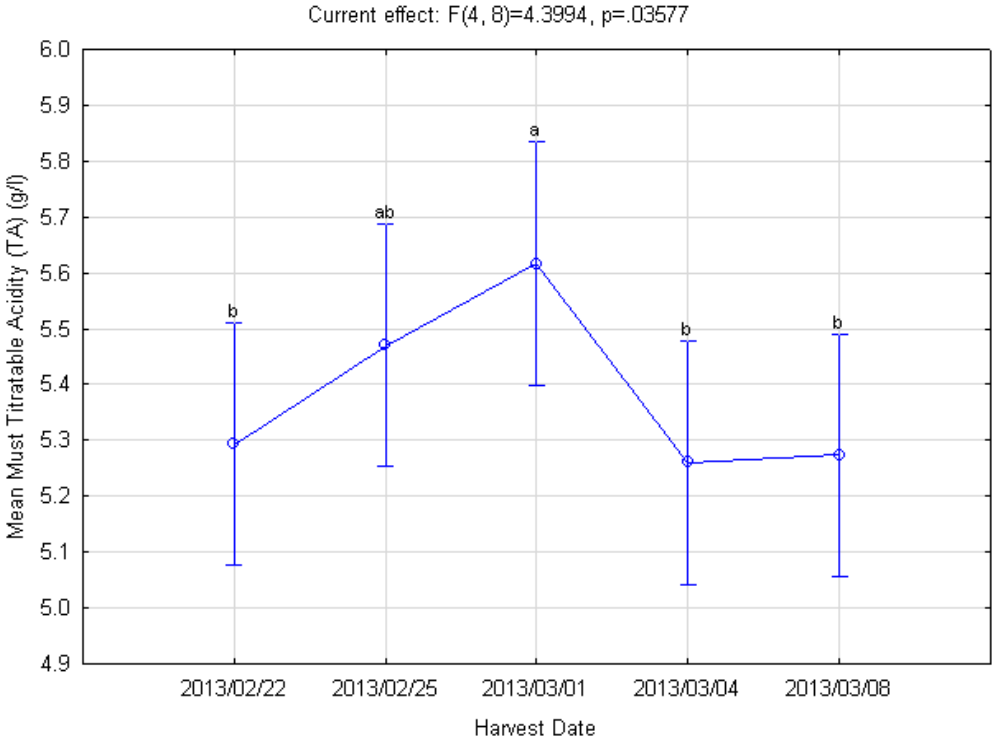

Figure B15: Mean must titratable acidity per harvest date

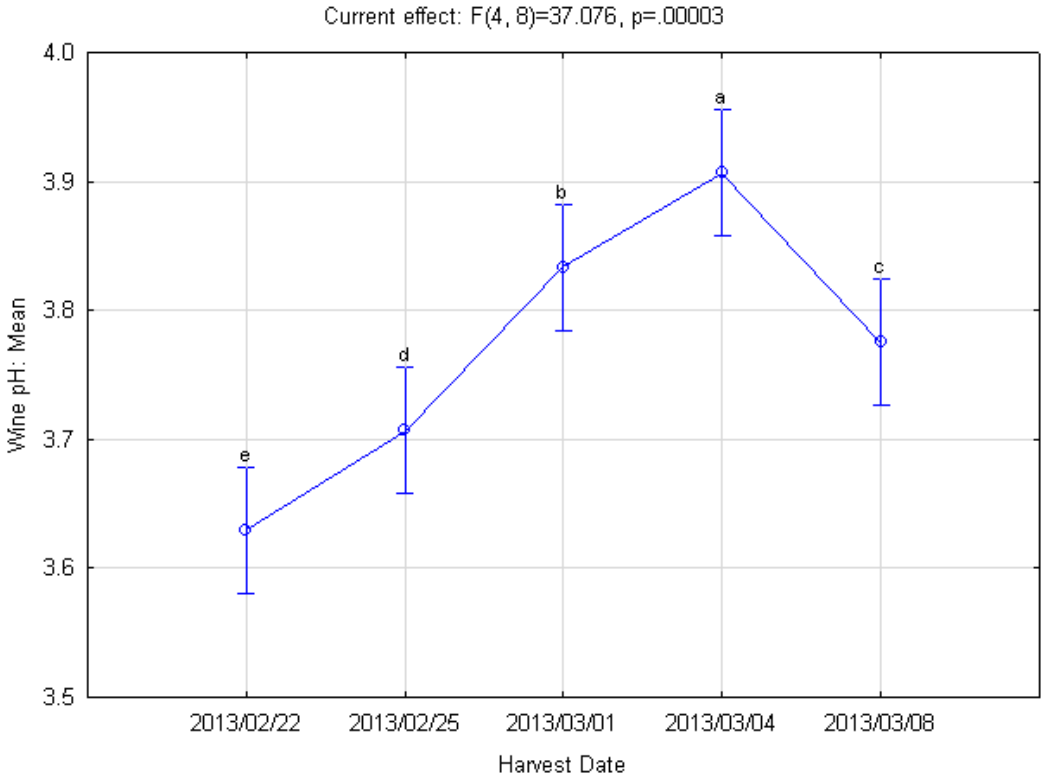

Figure B16: Mean wine pH per harvest date

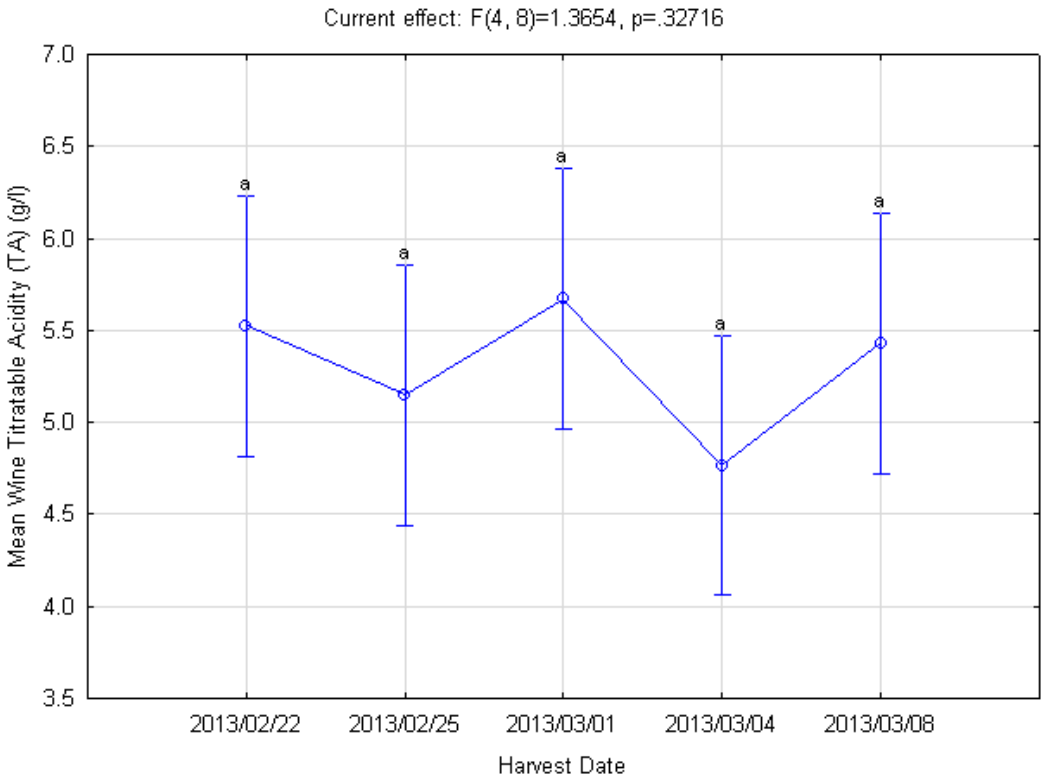

Figure B17: Mean wine titratable acidity per harvest date

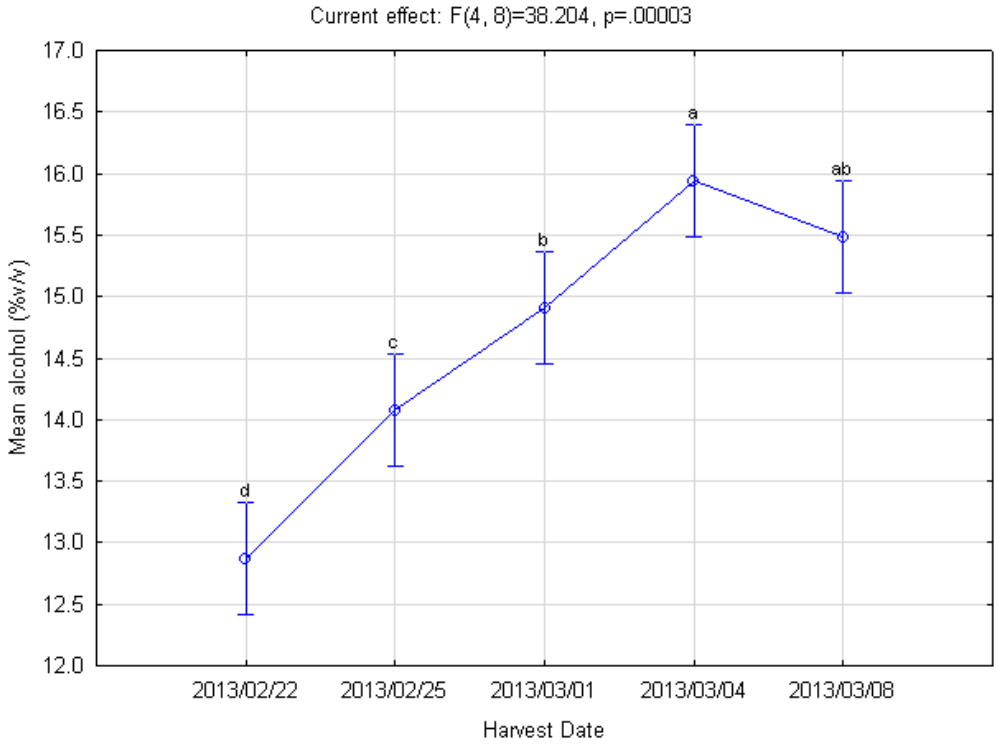

Figure B18: Mean alcohol per harvest date

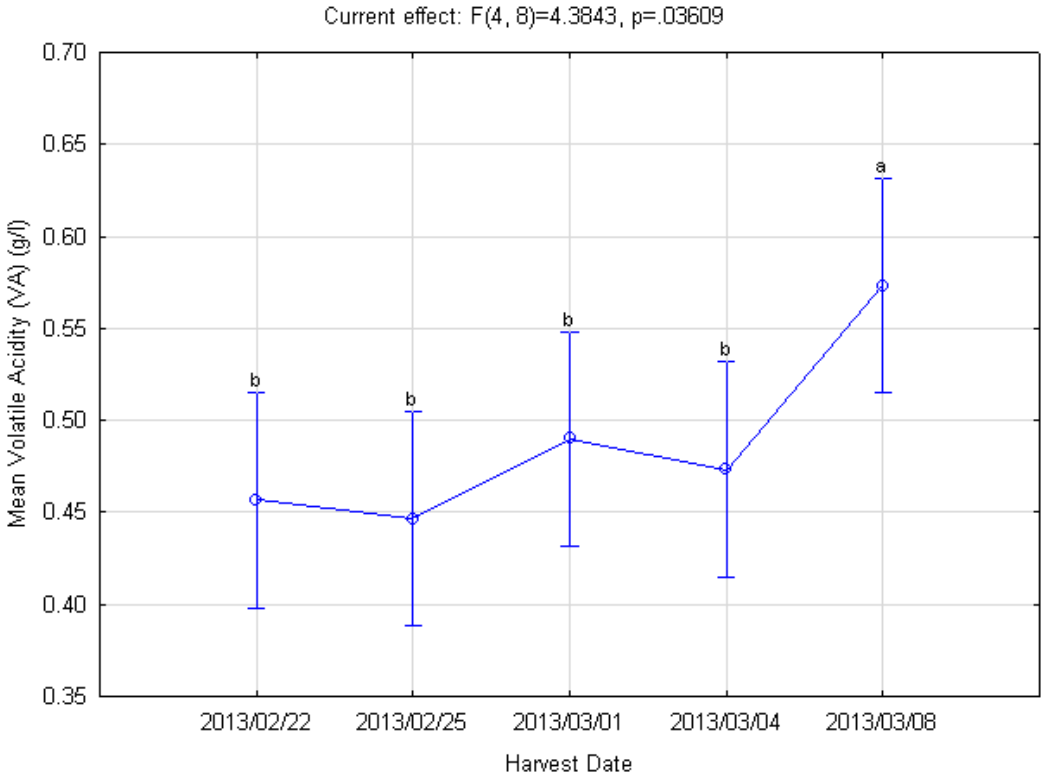

Figure B19: Mean volatile acidity per harvest date

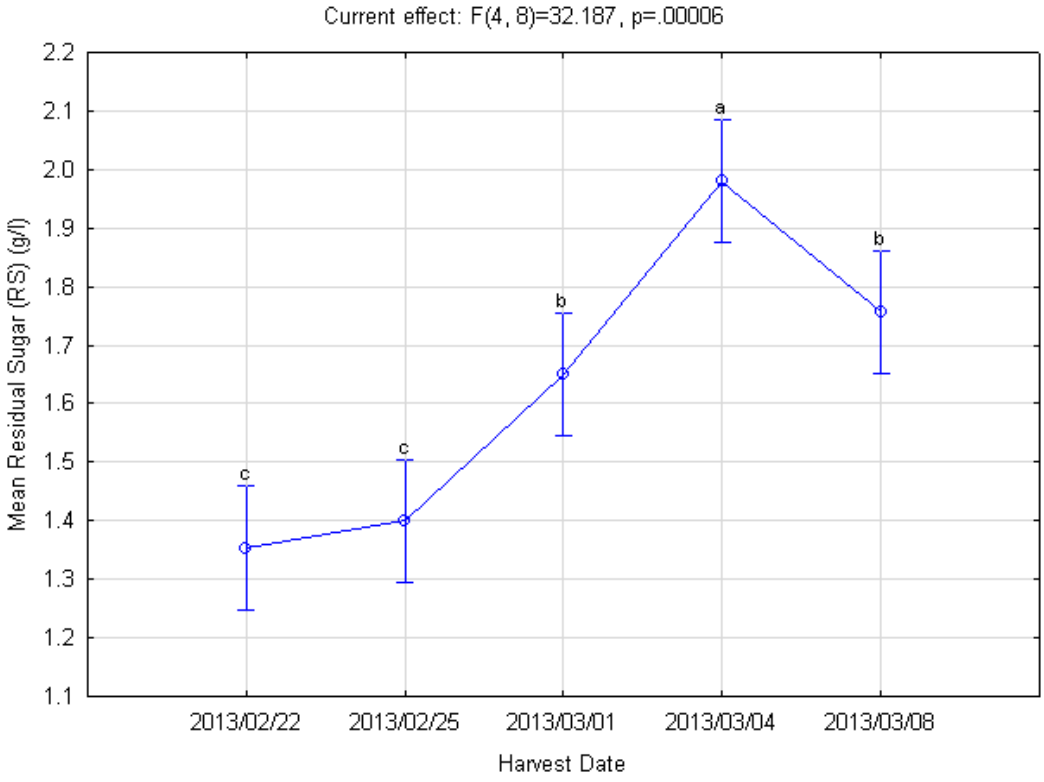

Figure B20: Mean residual sugar per harvest date

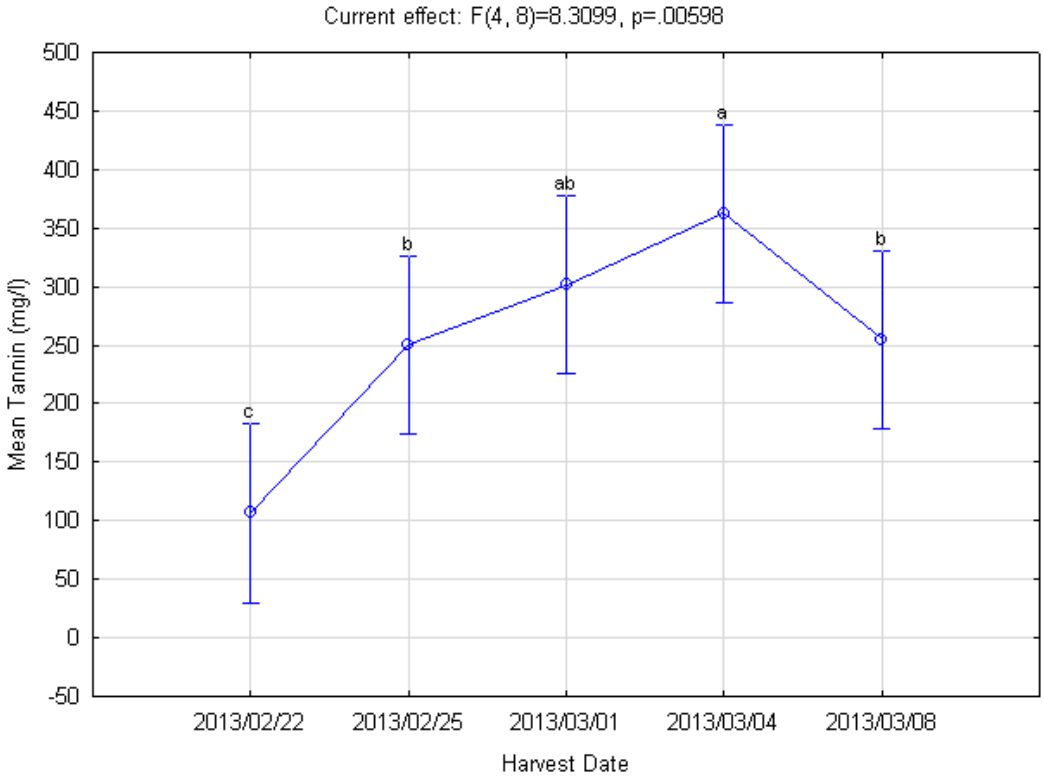

Figure B21: Mean tannin concentrations per harvest date

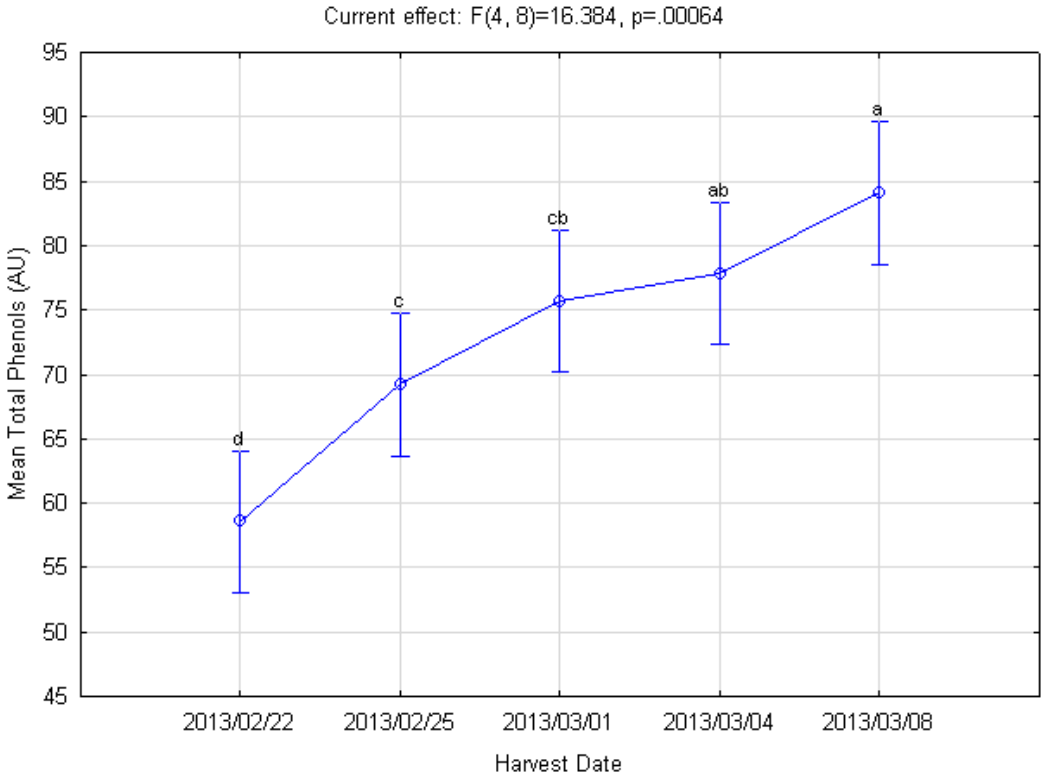

Figure B22: Mean total phenols per harvest date

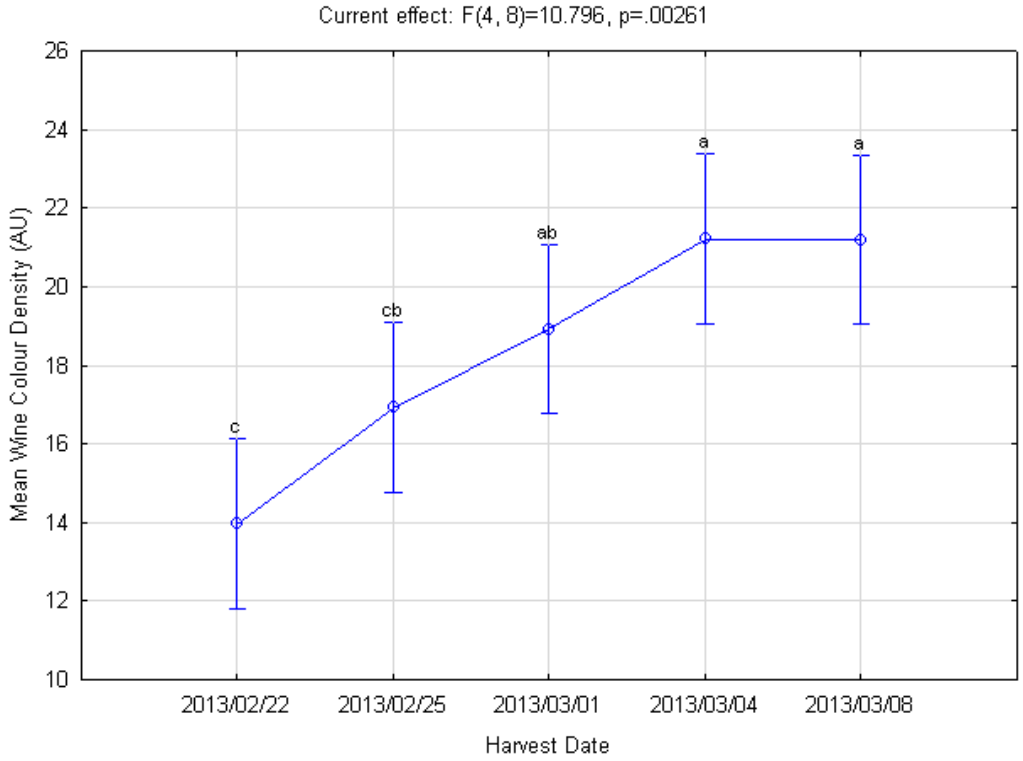

Figure B23: Mean wine colour density per harvest date

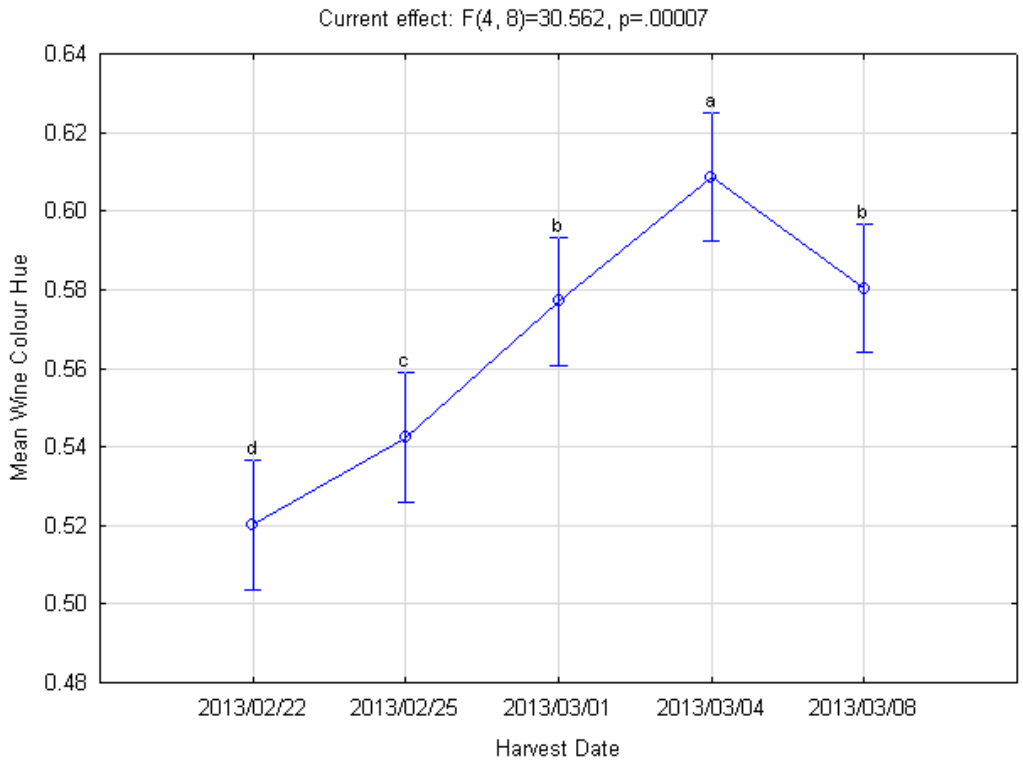

Figure B24: Mean wine colour hue per harvest date

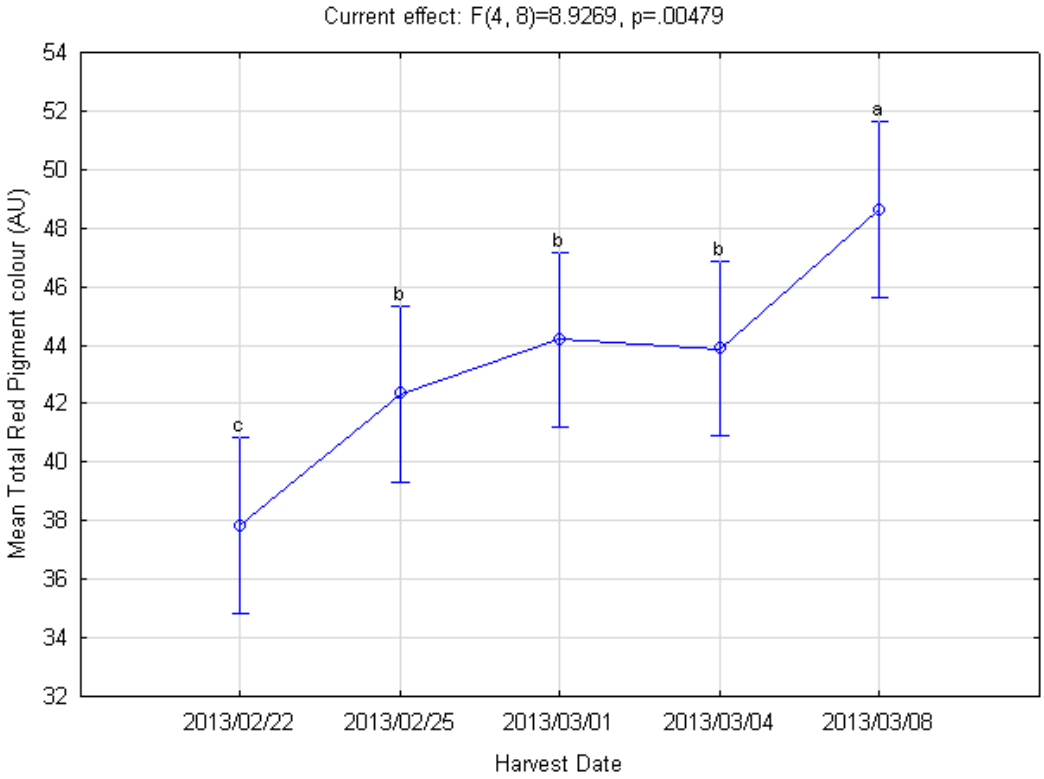

Figure B25: Mean total red pigment colour per harvest date

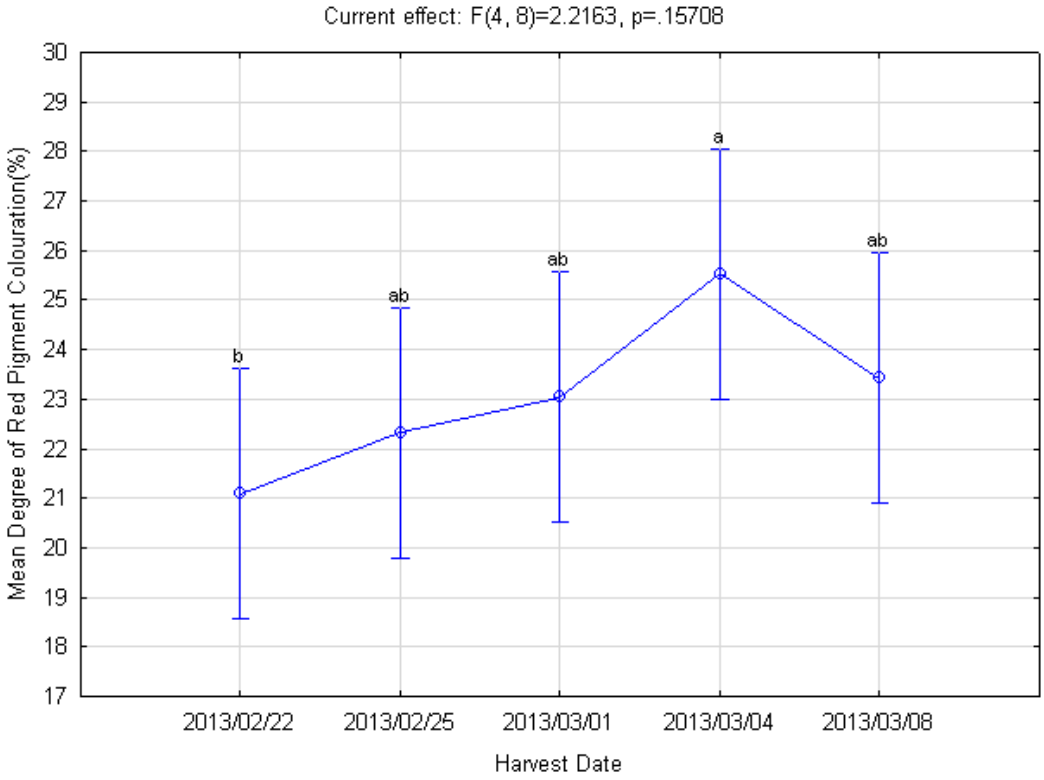

Figure B26: Mean degree of red pigment colouration per harvest date

Table B1 Results of the three-way ANOVA

	Sweetness	Sourness	Bitterness	Astringency	Alcohol
R^2	0.801	0.773	0.789	0.818	0.822
F	2.565	2.169	2.345	2.860	2.917
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Judge	9.456	7.904	7.596	9.862	11.769
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Wine	1.254	0.304	6.371	6.881	3.923
	0.292	0.875	0.000	< 0.0001	0.005
Repeat	2.924	1.035	10.946	23.383	6.165
	0.090	0.311	0.001	< 0.0001	0.014
Judge*Wine	0.757	1.009	0.930	1.059	1.047
	0.945	0.480	0.660	0.371	0.397
Judge*Repeat	3.285	1.539	2.051	2.268	1.424
	< 0.0001	0.046	0.002	0.001	0.084
Wine*Repeat	0.676	0.487	1.279	0.251	0.305
	0.610	0.745	0.282	0.909	0.874

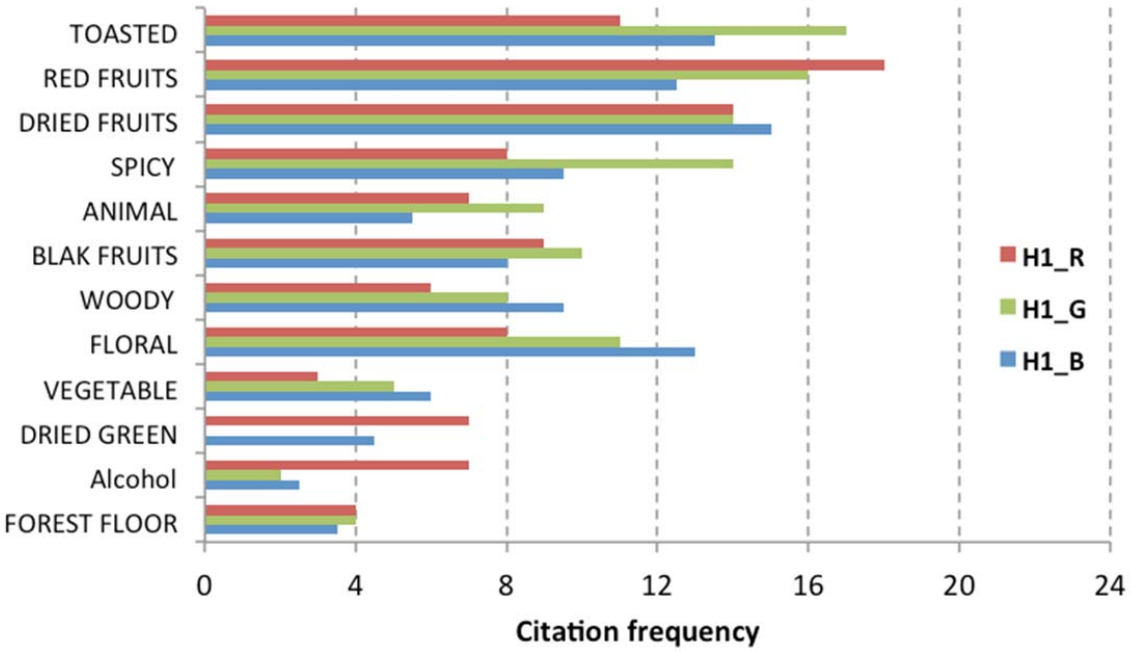

Figure B27: Harvest 1 attributes

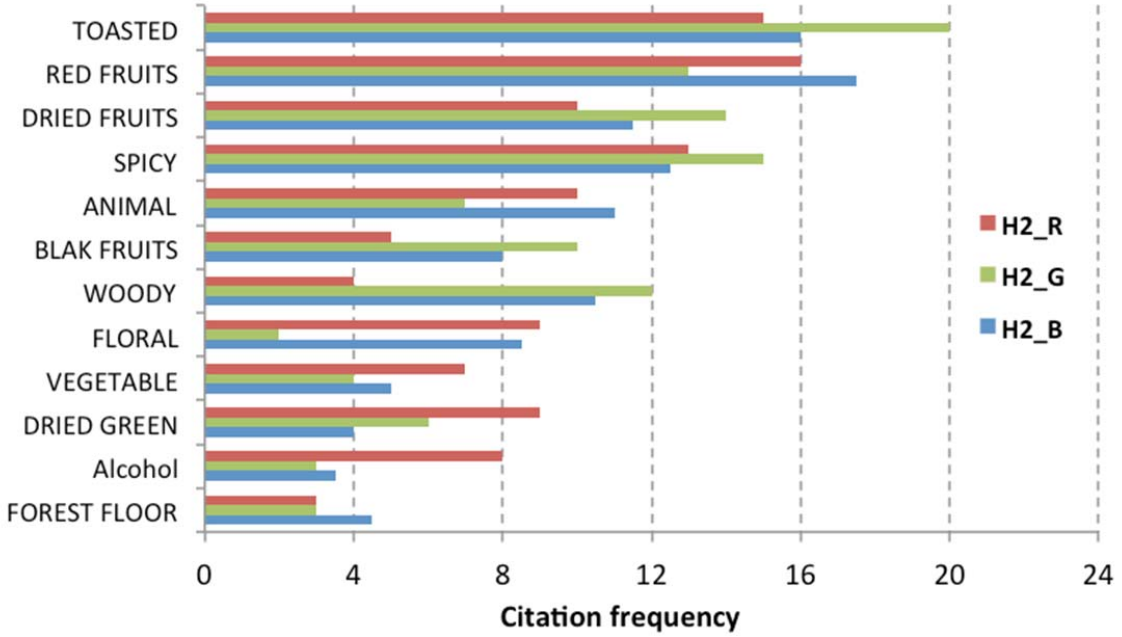

Figure B28: Harvest 2 attributes

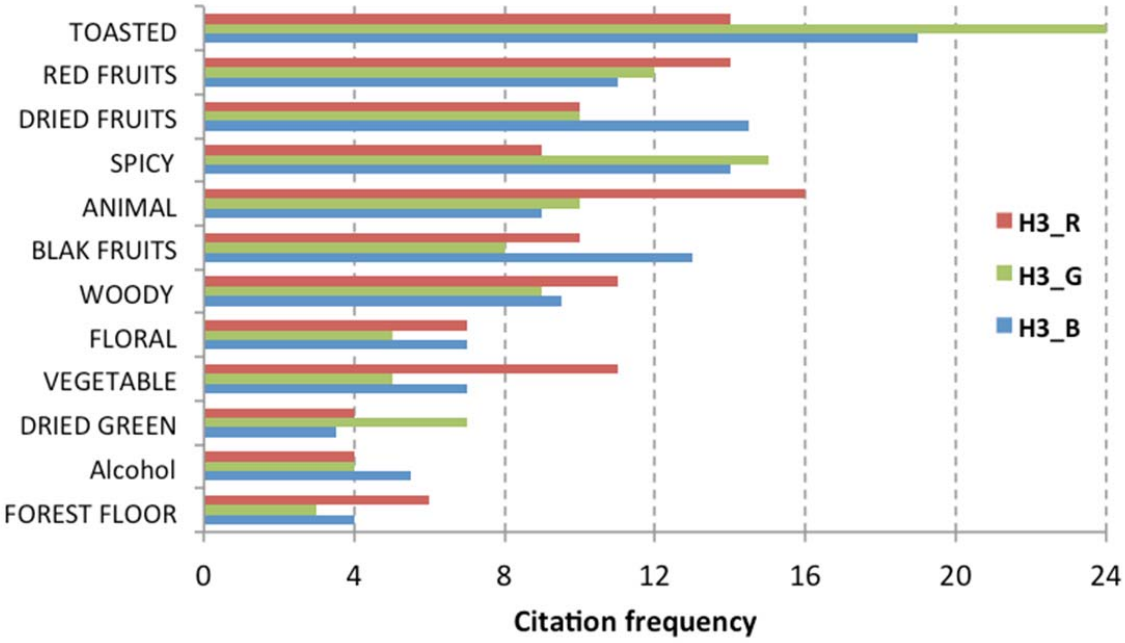

Figure B29: Harvest 3 attributes

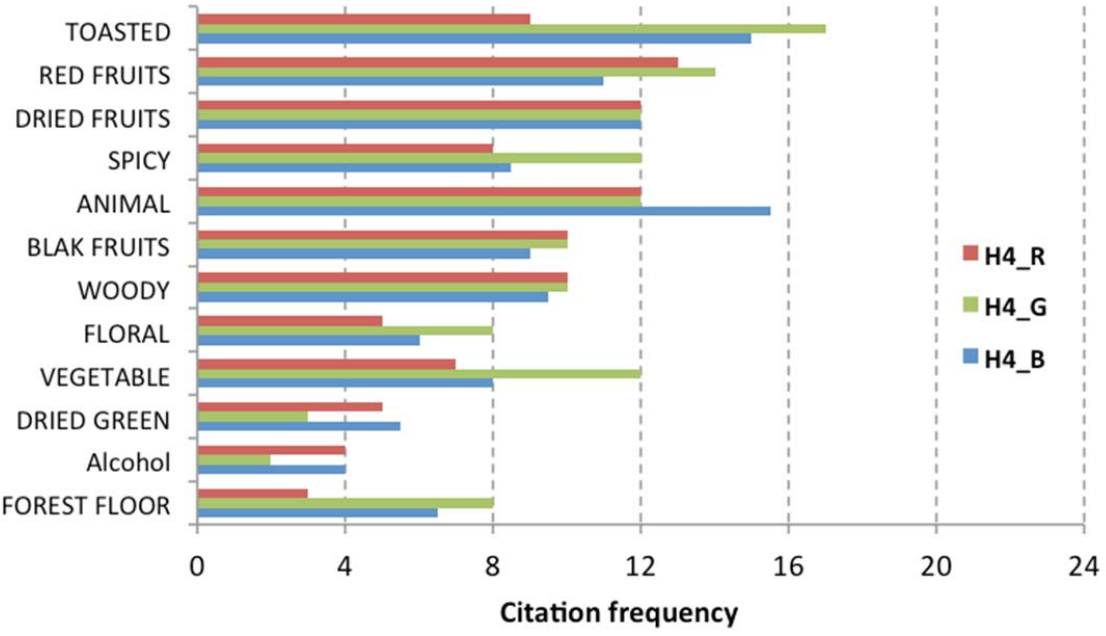

Figure B30: Harvest 4 attributes

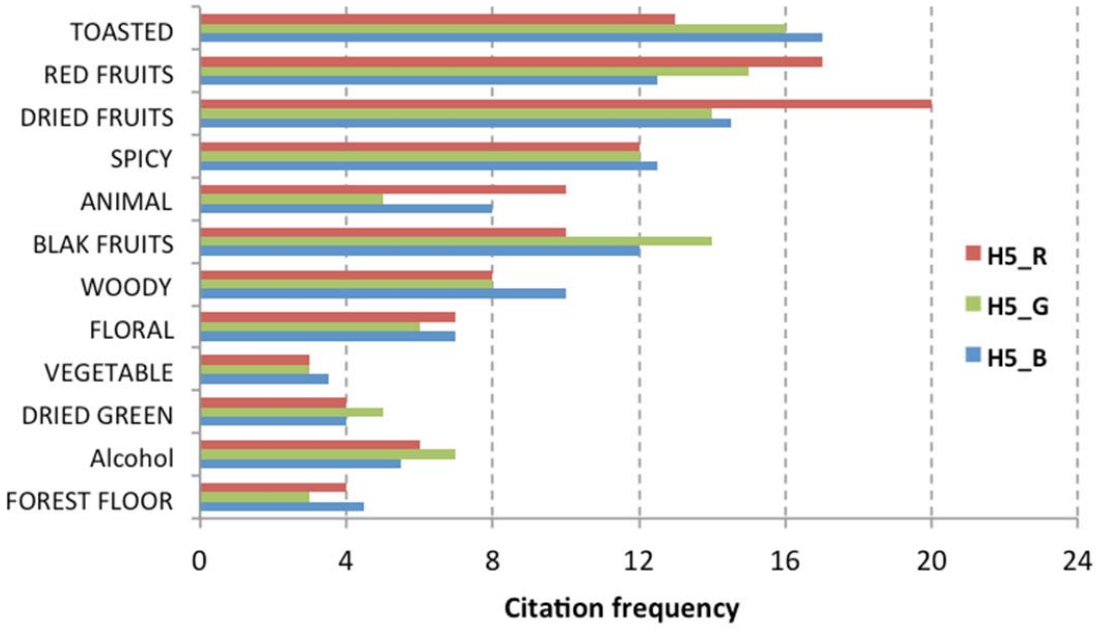

Figure B31: Harvest 5 attributes

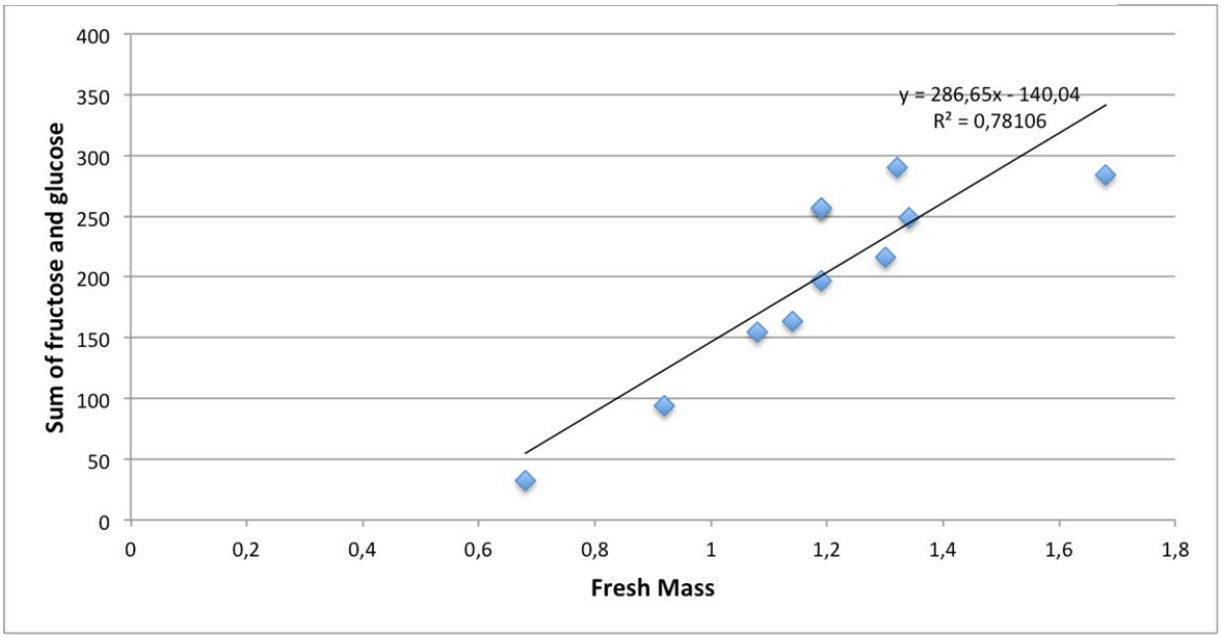

Figure B32: Correlation between the sum of glucose and fructose and berry fresh mass

Appendix C

1. SWP and PLWP results

Table C1: Mean SWP and PLWP (where values in red are higher than the desired threshold of vine water status)

Time period	Nov- Dec- 11		Jan-12		Feb-12		Mar-12	
	high vigour	low vigour	high vigour	low vigour	high vigour	low vigour	high vigour	low vigour
Mean SWP	539.7	739.3	687.0	862.9	1078.0	1065.8	1337.5	1266.7
Std Deviation SWP	189.3	246.5	219.7	210.6	256.8	215.8	157.6	179.5
Coefficient of variation SWP	35.1	33.3	32.0	24.4	23.8	20.3	11.8	14.2
Mean PLWP			355.3	368.9	470.7	489.9	729.9	719.3
Std Deviation PLWP			106.0	129.2	129.3	160.5	154.0	222.5
Coefficient of variation PLWP			29.8	35.0	27.5	32.8	21.1	30.9

Table C2: Mean SWP and PLWP of January per panel. (The mean for each vigour classification is presented in bold)

Time period: January

Panel number	n	Mean SWP (-kPa)	Std Deviation	Coeff Var (%)
5	8	931.25	193.5	20.7
6	7	892.86	262.4	29.4
7	8	837.50	198.0	23.6
8	8	793.75	154.9	19.5
low vigour	**31**	**862.90**	**210.5**	24.4
9	8	825	192.0	23.3
10	8	681.25	220.7	32.4
na (buffer panel)	**16**	**753.13**	**219**	29.1
11	7	607.14	147.4	24.2
12	7	687.14	222.6	32.4
13	8	731.25	298.9	40.9
14	8	712.5	145.2	20.4
high vigour	**30**	**687**	**219.7**	32

Table C2 (cont.)

Time period: January

Panel number	n	Mean PLWP (-kPa)	Std Deviation	Coeff Var (%)
5	46	392.17	141.88	36.18
6	46	373.26	111.12	29.77
7	46	364.57	121.04	33.20
8	46	345.65	136.06	39.36
low vigour	184	368.91	129.19	35.02
9	46	361.52	115.93	32.07
10	46	407.83	121.33	29.75
na (buffer panel)	92	384.67	120.90	31.43
11	46	348.70	94.63	27.14
12	46	361.96	114.05	31.51
13	46	378.91	111.16	29.34
14	46	331.74	97.18	29.30
high vigour	184	355.33	106.02	29.84

2. Sugar accumulation

Table C3: Results of estimated sugar per berry and fresh mass (SL curves)of Low vigour and High Vigour sections used to determine the keypoint and harvest dates.

Low Vigour

Degree days	Date	Brix	Fresh mass/berry	Sugar/berry (mg)	Sugar(mg)/berry/day
947.4	12/01/12	12.69	0.82	104.50	
1081.2	12/01/17	14.72	0.88	129.48	5.00
1237.9	12/01/23	15.56	1.12	174.95	7.58
1420.6	12/01/30	19.14	1.10	210.62	5.10
1514.6	12/02/03	17.99	1.04	187.81	-5.70
(KP) 1602.6	**12/02/06**	**20.70**	**1.13**	**235.70**	**15.96**
1673.0	12/02/09	20.7	1.19	247.69	4.00
1791.5	12/02/15	20.3	1.32	269.32	3.61
1836.4	12/02/17	22.85	1.28	293.59	12.13
1937.0	12/02/21	21.7	1.41	306.72	3.28
2072.1	12/02/27	23.55	1.44	339.90	5.53
2139.9	12/03/01	25.7	1.17	301.05	-12.95
2228.5	12/03/05	24.7	1.12	276.23	-6.20
2302.9	12/03/09	27.35	1.10	302.99	6.69

Table C4: (cont.)

High Vigour

Degree days	Date	Brix	Fresh mass/berry	Sugar/berry (mg)	Sugar(mg)/berry/day
947.4	12/01/12	11.05	1.17	129.67	
1081.2	12/01/17	12.61	1.27	160.50	6.17
1237.9	12/01/23	13.96	1.45	203.03	7.09
1420.6	12/01/30	16.51	1.52	251.54	6.93
1514.6	12/02/03	16.09	1.57	253.41	0.47
1602.6	**12/02/06**	**17.93**	**1.53**	**276.10**	**7.56**
1673.0	12/02/09	18.85	1.72	324.63	16.18
1791.5	12/02/15	18.5	1.72	318.88	-0.96
1836.4	12/02/17	22	1.52	336,40	8.76
1937.0	12/02/21	22.95	1.40	321.23	-3.79
2072.1	12/02/27	23.3	1.56	365.51	7.38
2139.9	12/03/01	24.3	1.61	391.83	8.78
2228.5	12/03/05	24	1.48	356.02	-8.95
2302.9	12/03/09	24.55	1.51	372.8	4.16

Table C5: Evolution of the sum of glucose and fructose per berry (Average of HV and LV)

Date	Sum of glucose and fructose (per berry)
2014/02/03	187.2^{c}
2014/02/06	242.0^{cb}
2014/02/15	257.9^{ac}
2014/02/17	234.6^{cb}
2014/02/21	288.5^{ab}
2014/03/01	332.4^{a}
2014/03/09	332.7^{a}

Table C6: High vigour fresh mass and sugar per berry (sum of glucose and fructose (mg/berry))

	Sum of glucose and fructose per berry (mg/berry)	Fresh Mass (g)
12/02/03	220	1.57
12/02/06	274	1.53
12/02/15	262	1.72
12/02/17	264	1.52
12/02/21	309	1.39
12/03/01	383	1.61
12/03/09	417	1.51

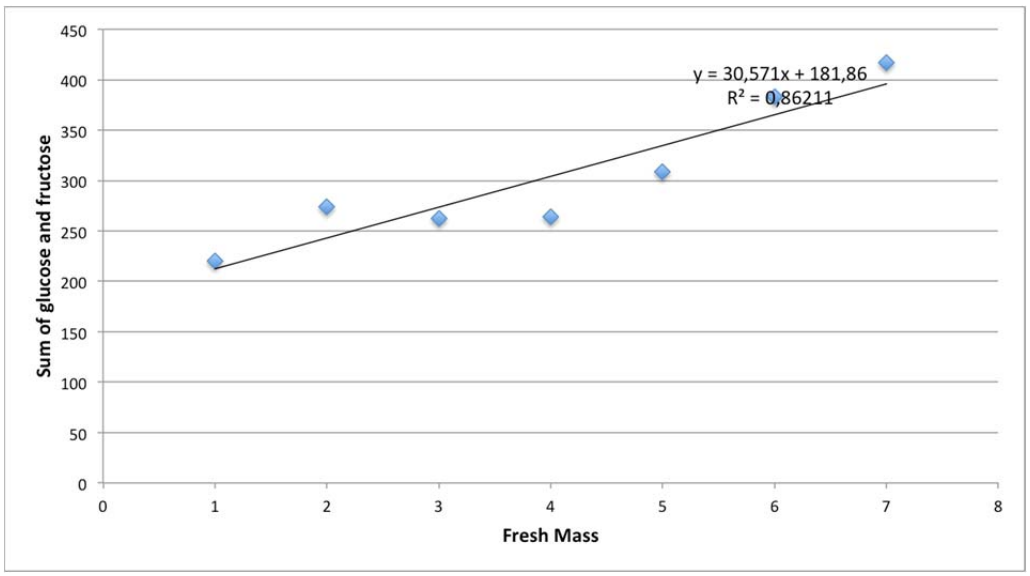

Figure C1: Correlation between the sum of glucose and fructose and berry fresh mass for high vigour vines

Table C7: Low vigour fresh mass and sugar per berry (sum of glucose and fructose (mg/berry))

	Sum of glucose and fructose per berry (mg/berry)	Fresh Mass (g)
12/02/03	154	1.04
12/02/06	210	1.13
12/02/15	254	1.32
12/02/17	205	1.28
12/02/21	268	1.41
12/03/01	282	1.17
12/03/09	248	1.10

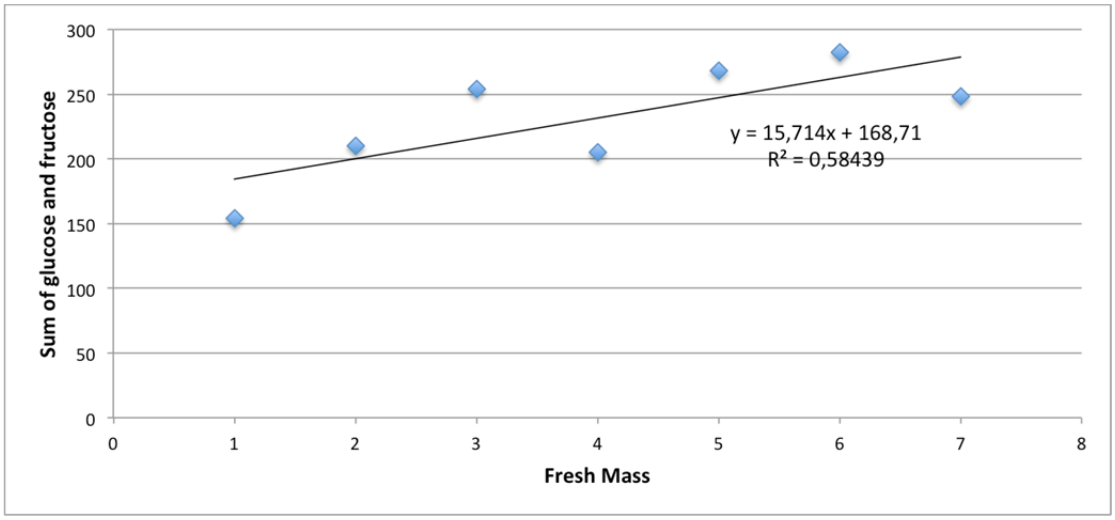

Figure C2: Correlation between the sum of glucose and fructose and berry fresh mass for low vigour vines